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<b>(54) Title:</b> CDR-GRAFTED ANTI-TISSUE FACTOR ANTIBODIES AND METHODS OF USE THEREOF			
<b>(57) Abstract</b> <p>The present invention provides CDR-grafted antibodies against human tissue factor that retain the high binding affinity of rodent monoclonal antibodies against tissue factor but have reduced immunogenicity. The present humanized antibodies are potent anticoagulants and are thus useful in the treatment and prophylaxis of human thrombotic disease. The invention also provides methods of making the CDR-grafted antibodies and pharmaceutical compositions for the attenuation or prevention of coagulation.</p>			

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CDR-GRAFTED ANTI-TISSUE FACTOR  
ANTIBODIES AND METHODS OF USE THEREOF

FIELD OF THE INVENTION

5           Monoclonal antibodies capable of inhibiting  
tissue factor (TF) are useful as anticoagulants.  
Conventional rodent monoclonal antibodies, however, have  
limited use in human therapeutic and diagnostic  
10 applications due to immunogenicity and short serum half-  
life. The present invention provides CDR-grafted  
monoclonal antibodies against TF that retain the high  
binding affinity of rodent antibodies but have reduced  
immunogenicity. The present humanized antibodies are  
15 potent anticoagulants and are thus useful in the  
treatment and prophylaxis of human thrombotic disease.  
The invention also provides methods of making the CDR-  
grafted antibodies and pharmaceutical compositions for  
the attenuation or prevention of coagulation.

20 BACKGROUND OF THE INVENTION

          The coagulation of blood involves a cascading  
series of reactions leading to the formation of fibrin.  
The coagulation cascade consists of two overlapping  
25 pathways, both of which are required for hemostasis.  
The intrinsic pathway comprises protein factors present  
in circulating blood, while the extrinsic pathway  
requires tissue factor, which is expressed on the cell  
surface of a variety of tissues in response to vascular  
30 injury. Davie et al., 1991, Biochemistry 30:10363.  
Agents that interfere with the coagulation cascade, such

as heparin and coumarin derivatives, have well-known  
1 therapeutic uses in the prophylaxis of venous  
thrombosis. Goodman and Gilman, eds., 1980, The  
Pharmacological Basis of Therapeutics, MacMillan  
Publishing Co., Inc., New York.

5 Tissue factor (TF) has been investigated as a  
target for anticoagulant therapy. TF is a membrane  
glycoprotein that functions as a receptor for factor VII  
and VIIa and thereby initiates the extrinsic pathway of  
the coagulation cascade in response to vascular injury.  
10 In addition to its role in the maintenance of hemostasis  
by initiation of blood clotting, TF has been implicated  
in pathogenic conditions. Specifically, the synthesis  
and cell surface expression of TF has been implicated in  
vascular disease (Wilcox et al., 1989, Proc. Natl. Acad.  
15 Sci. 86:2839) and gram-negative septic shock (Warr et  
al., 1990, Blood 75:1481).

Ruf et al. (1991, Thrombosis and Haemostasis  
66:529) characterized the anticoagulant potential of  
murine monoclonal antibodies against human TF. The  
20 inhibition of TF function by most of the monoclonal  
antibodies that were assessed was dependent upon the  
dissociation of the TF/VIIa complex that is rapidly  
formed when TF contacts plasma. Such antibodies were  
thus relatively slow inhibitors of TF in plasma. One  
25 monoclonal antibody, TF8-5G9, was capable of inhibiting  
the TF/VIIa complex without dissociation of the complex,  
thus providing an immediate anticoagulant effect in  
plasma. Ruf et al. suggest that mechanisms that  
inactivate the TF/VIIa complex, rather than prevent its  
30 formation, may provide strategies for interruption of  
coagulation in vivo.

The therapeutic use of monoclonal antibodies  
1 against TF is limited in that currently available  
monoclonals are of rodent origin. The use of rodent  
antibodies in human therapy presents numerous problems,  
the most significant of which is immunogenicity.  
5 Repeated doses of rodent monoclonal antibodies have been  
found to elicit an anti-immunoglobulin response termed  
human anti-mouse antibody (HAMA), which can result in  
immune complex disease and/or neutralization of the  
therapeutic antibody. See, e.g., Jaffers et al. (1986)  
10 Transplantation 41:572. While the use of human  
monoclonal antibodies would address this limitation, it  
has proven difficult to generate large amounts of human  
monoclonal antibodies by conventional hybridoma  
technology.  
15 Recombinant technology has been used in an  
effort to construct "humanized" antibodies that maintain  
the high binding affinity of rodent monoclonal  
antibodies but exhibit reduced immunogenicity in humans.  
Chimeric antibodies have been produced in which the  
20 variable (V) region of a mouse antibody is combined with  
the constant (C) region of a human antibody in an effort  
to maintain the specificity and affinity of the rodent  
antibody but reduce the amount of protein that is non-  
human and thus immunogenic. While the immune response  
25 to chimeric antibodies is generally reduced relative to  
the corresponding rodent antibody, the immune response  
cannot be completely eliminated, because the mouse V  
region is capable of eliciting an immune response.  
Lobuglio et al. (1989) Proc. Natl. Acad. Sci. 86:4220;  
30 Jaffers et al. (1986) Transplantation 41:572.

In a recent approach to reducing  
1 immunogenicity of rodent antibodies, only the rodent  
complementarity determining regions (CDRs), rather than  
the entire V domain, are transplanted to a human  
antibody. Such humanized antibodies are known as CDR-  
5 grafted antibodies. CDRs are regions of  
hypervariability in the V regions that are flanked by  
relatively conserved regions known as framework (FR)  
regions. Each V domain contains three CDRs flanked by  
four FRs. The CDRs fold to form the antigen binding  
10 site of the antibody, while the FRs support the  
structural conformations of the V domains. Thus by  
transplanting the rodent CDRs to a human antibody, the  
antigen binding domain can theoretically also be  
transferred. Owens et al. (1994) J. Immunol. Methods  
15 168:149 and Winter et al. (1993) Immunology Today 14:243  
review the development of CDR-grafted antibodies.

Orlandi et al. (1989) Proc. Natl. Acad. Sci.  
USA 86:3833 constructed a humanized antibody against the  
relatively simple hapten nitrophenacetyl (NP). The CDR-  
20 grafted antibody contained mouse CDRs and human FRs, and  
exhibited NP binding activity similar to the native  
mouse antibody. However, the construction of CDR-  
grafted antibodies recognizing more complex antigens has  
resulted in antibodies having binding activity  
25 significantly lower than the native rodent antibodies.  
In numerous cases it has been demonstrated that the mere  
introduction of rodent CDRs into a human antibody  
background is insufficient to maintain full binding  
activity, perhaps due to distortion of the CDR  
30 conformation by the human FR.

For example, Gorman et al. (1991) Proc. Natl. Acad. Sci. 88:4181 compared two humanized antibodies against human CD4 and observed considerably different avidities depending upon the particular human framework region of the humanized antibody. Co et al. (1991) Proc. Natl. Acad. Sci. USA 88:2869 required a refined computer model of the murine antibody of interest in order to identify critical amino acids to be considered in the design of a humanized antibody. Kettleborough et al. (1991) Protein Engineering 4:773 report the influence of particular FR residues of a CDR-grafted antibody on antigen binding, and propose that the residues may directly interact with antigen, or may alter the conformation of the CDR loops. Similarly, Singer et al. (1993) J. Immunol. 150:2844 report that optimal humanization of an anti-CD18 murine monoclonal antibody is dependent upon the ability of the selected FR to support the CDR in the appropriate antigen binding conformation. Accordingly, recreation of the antigen-binding site requires consideration of the potential intrachain interactions between the FR and CDR, and manipulation of amino acid residues of the FR that maintain contacts with the loops formed by the CDRs. While general theoretical guidelines have been proposed for the design of humanized antibodies (see, e.g., Owens et al.), in all cases the procedure must be tailored and optimized for the particular rodent antibody of interest.

There is a need in the art for humanized antibodies with reduced immunogenicity and comparable binding affinity relative to the parent rodent antibody for various therapeutic applications. In particular,

there is a need for a humanized antibody against human  
1 tissue factor having anticoagulant activity and useful  
in the treatment and prevention of thrombotic disease.

#### SUMMARY OF THE INVENTION

5

The present invention is directed to CDR-grafted antibodies capable of inhibiting human tissue factor wherein the CDRs are derived from a non-human monoclonal antibody against tissue factor and the FR and  
10 constant (C) regions are derived from one or more human antibodies. In a preferred embodiment, the murine monoclonal antibody is TF8-5G9.

In another embodiment, the present invention provides a method of producing a CDR-grafted antibody  
15 capable of inhibiting human tissue factor which method comprises constructing one or more expression vectors containing nucleic acids encoding CDR-grafted antibody heavy and light chains, transfecting suitable host cells with the expression vector or vectors, culturing the  
20 transfected host cells, and recovering the CDR-grafted antibody.

The present invention also provides a method of attenuation of coagulation comprising administering a CDR-grafted antibody capable of inhibiting human tissue  
25 factor to a patient in need of such attenuation.

The present invention further provides a method of treatment or prevention of thrombotic disease comprising administering a CDR-grafted antibody capable of inhibiting human tissue factor to a patient in need  
30 of such treatment or prevention. In a preferred



embodiment, the thrombotic disease is intravascular  
1 coagulation, arterial restenosis or arteriosclerosis.

Another embodiment of the present invention is  
directed to a pharmaceutical composition comprising CDR-  
grafted antibodies capable of inhibiting human tissue  
5 factor and further comprising a pharmaceutically  
acceptable carrier.

#### BRIEF DESCRIPTION OF THE DRAWINGS

10 Fig. 1 provides the nucleotide and deduced  
amino acid sequences of the heavy chain of murine  
monoclonal antibody TF8-5G9.

Fig. 2 provides the nucleotide and deduced  
amino acid sequences of the light chain of murine  
15 monoclonal antibody TF8-5G9.

Fig. 3 is a graph depicting the ability of  
CDR-grafted antibody TF8HCDR1 x TF8LCDR1 to bind to  
human tissue factor and to compete with murine  
monoclonal antibody TF85G9 for binding to tissue factor.  
20 Solid symbols indicate direct binding of TF8HCDR1 x  
TF8LCDR1 and the positive control chimeric TF85G9 to  
tissue factor. Open symbols indicate competition  
binding of TF8HCDR1 x TF8LCDR1 or chimeric TF85G9 with  
murine monoclonal antibody TF85G9.

25 Fig. 4 presents the DNA sequence of expression  
vector pEe6TF8HCDR20 and the amino acid sequence of the  
coding regions of the CDR-grafted heavy chain TF8HCDR20.

Fig. 5 presents the DNA sequence of expression  
vector pEe12TF8LCDR3 and the amino acid sequence of the  
30 coding regions of the CDR-grafted light chain TF8LCDR3.

Fig. 6 is a graph depicting the ability of  
1 CDR-grafted antibody TF8HCDR20 x TF8LCDR3 to bind to  
human tissue factor.

Fig. 7 is a graph depicting the ability of  
CDR-grafted antibody TF8HCDR20 x TF8LCDR3 to compete  
5 with murine monoclonal antibody TF85G9 for binding to  
tissue factor.

Fig. 8 is a graph depicting the ability of  
CDR-grafted antibody TF8HCDR20 x TF8LCDR3 to inhibit  
factor X activation.

10 Fig. 9 provides expression vector  
pEe6TF8HCDR20 resulting from the subcloning of CDR-  
grafted heavy chain TF8HCDR20 into myeloma expression  
vector pEehCMV-BglI. The following abbreviations are  
used: VH is the CDR-grafted heavy chain variable  
15 region; Cy4 is the human IgG4 constant region; pA is the  
polyadenylation signal; ampR is the  $\beta$ -lactamase gene;  
and hCMV is human cytomegalovirus.

Fig. 10 provides expression vector  
pEel2TF8LCDR3 resulting from the subcloning of CDR-  
20 grafted light chain TF8LCDR3 into myeloma expression  
vector pEel2. The following abbreviations are used: VL  
is the CDR-grafted light chain variable region; CK is  
the human kappa constant region; SVE is the SV40 early  
promoter; GS is glutamine synthetase cDNA. Other  
25 abbreviations are as noted in Fig. 9.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention provides CDR-grafted  
30 antibodies capable of inhibiting human tissue factor  
wherein the CDRs are derived from a non-human monoclonal

antibody against tissue factor and the FR and C regions  
1 are derived from one or more human antibodies. The  
present invention further provides methods of making and  
using the subject CDR-grafted antibodies.

In accordance with the present invention, the  
5 CDR-grafted antibody is an antibody in which the CDRs  
are derived from a non-human antibody capable of binding  
to and inhibiting the function of human tissue factor,  
and the FR and C regions of the antibody are derived  
from one or more human antibodies. The CDRs derived  
10 from the non-human antibody preferably have from about  
90% to about 100% identity with the CDRs of the non-  
human antibody, although any and all modifications,  
including substitutions, insertions and deletions, are  
contemplated so long as the CDR-grafted antibody  
15 maintains the ability to bind to and inhibit tissue  
factor. The regions of the CDR-grafted antibodies that  
are derived from human antibodies need not have 100%  
identity with the human antibodies. In a preferred  
embodiment, as many of the human amino acid residues as  
20 possible are retained in order that immunogenicity is  
negligible, but the human residues, in particular  
residues of the FR region, are substituted as required  
and as taught hereinbelow in accordance with the present  
invention. Such modifications as disclosed herein are  
25 necessary to support the antigen binding site formed by  
the CDRs while simultaneously maximizing the  
humanization of the antibody.

Non-human monoclonal antibodies against human  
tissue factor from which the CDRs can be derived are  
30 known in the art (Ruf et al., 1991; Morrissey et al.,  
1988, Thrombosis Research 52:247) or can be produced by

well-known methods of monoclonal antibody production  
1 (see, e.g. Harlow et al., eds., 1988, Antibodies, A  
Laboratory Manual, Cold Spring Harbor Laboratories, Cold  
Spring Harbor, New York). Purified human tissue factor  
against which monoclonal antibodies can be raised is  
5 similarly well-known (Morrissey et al., 1987, Cell  
50:129) and available to the skilled artisan. Murine  
monoclonal antibodies, and in particular murine  
monoclonal antibody TF8-5G9 disclosed by Ruf et al. and  
Morrissey et al., 1988, Thrombosis Research 52:247, and  
10 U.S. Patent No. 5,223,427 are particularly preferred.

The ordinarily skilled artisan can determine  
the sequences of the CDRs by reference to published  
scientific literature or sequence databanks, or by  
cloning and sequencing the heavy and light chains of the  
15 antibodies by conventional methodology. In accordance  
with the present invention, the cDNA and amino acid  
sequences of the heavy chain (SEQ ID NOS:1 and 2,  
respectively) and light chain (SEQ ID NOS:3 and 4,  
respectively) of murine monoclonal antibody TF8-5G9 are  
20 provided. The cDNA and deduced amino acid sequence of  
the murine TF8-5G9 heavy chain is provided at Figure 1.  
The cDNA and deduced amino acid sequence of the murine  
TF8-5G9 light chain is provided at Figure 2.

Each of the heavy and light chain variable  
25 regions contain three CDRs that combine to form the  
antigen binding site. The three CDRs are surrounded by  
four FR regions that primarily function to support the  
CDRs. The sequences of the CDRs within the sequences of  
the variable regions of the heavy and light chains can  
30 be identified by computer-assisted alignment according  
to Kabat et al. (1987) in Sequences of Proteins of

Immunological Interest, 4th ed., United States

- 1 Department of Health and Human Services, US Government  
Printing Office, Washington, D.C., or by molecular  
modeling of the variable regions, for example utilizing  
the ENCAD program as described by Levitt (1983) J. Mol.  
5 Biol. 168:595.

In a preferred embodiment the CDRs are derived  
from murine monoclonal antibody TF8-5G9. The preferred  
heavy chain CDRs have the following sequences:

10	CDR1	DDYMH	(SEQ ID NO:5)
	CDR2	LIDPENGNTIYDPKFQG	(SEQ ID NO:6)
	CDR3	DNSYYFDY	(SEQ ID NO:7)

The preferred light chain CDRs have the following  
15 sequences:

	CDR1	KASQDIRKYLN	(SEQ ID NO:8)
	CDR2	YATSLAD	(SEQ ID NO:9)
	CDR3	LQHGESPYT	(SEQ ID NO:10)

20

The sequences of the CDRs of the murine or other non-  
human antibody, and in particular the sequences of the  
CDRs of TF8-5G9, may be modified by insertions,  
substitutions and deletions to the extent that the CDR-  
25 grafted antibody maintains the ability to bind to and  
inhibit human tissue factor. The ordinarily skilled  
artisan can ascertain the maintenance of this activity  
by performing the functional assays described  
hereinbelow. The CDRs can have, for example, from about  
30 50% to about 100% homology to the CDRs of SEQ ID NOS:5-  
10. In a preferred embodiment the CDRs have from about

35

80% to about 100% homology to the CDRs of SEQ ID NOS:5-10. In a more preferred embodiment the CDRs have from about 90% to about 100% homology to the CDRs of SEQ ID NOS:5-10. In a most preferred embodiment the CDRs have from about 100% homology to the CDRs of SEQ ID NOS:5-10.

The FR and C regions of the CDR-grafted antibodies of the present invention are derived from one or more human antibodies. Human antibodies of the same class and type as the antibody from which the CDRs are derived are preferred. The FR of the variable region of the heavy chain is preferably derived from the human antibody KOL (Schmidt et al., 1983, Hoppe-Seyler's Z. Physiol. Chem. 364:713) The FR of the variable region of the light chain is preferably derived from the human antibody REI (Epp et al., 1974, Eur. J. Biochem. 45:513). In accordance with the present invention, it has been discovered that certain residues of the human FR are preferably replaced by the corresponding residue of the non-human antibody from which the CDRs are derived. For example, certain FR residues of TF8-5G9 are preferably retained to achieve optimal binding to antigen.

For convenience, the numbering scheme of Kabat et al. has been adopted herein. Residues are designated by lower case numbers or hyphens as necessary to conform the present sequences to the standard Kabat numbered sequence.

In accordance with the present invention, residues that are retained in the FR region, i.e. residues that are not replaced by human FR residues, are determined according to the following guidelines. Residues that are idiosyncratic to the parent antibody,

- e.g. TF8-5G9, relative to a human consensus sequence of
- 1 Kabat et al, are retained. Residues of the parent antibody that are in agreement with the consensus sequence are retained if the corresponding residue of the human antibody, e.g. KOL or REI, is idiosyncratic.
  - 5 Residues that are part of the antibody loop canonical structures defined by Chothia et al. (1989) Nature 342:877, such as residue 71 of the heavy and light chains, are retained. FR residues predicted to form loops, such as residues 28-30 of the heavy chain, are
  - 10 retained. FR residues predicted to influence the conformation of the CDRs such as residues 48 and 49 preceding CDR2 of the heavy chain, are retained. Residues that have been demonstrated to be critical in the humanization of other antibodies may also be
  - 15 retained. The foregoing guidelines are followed to the extent necessary to support the antigen binding site formed by the CDRs while simultaneously maximizing the humanization of the antibody.

The amino acid sequence of a representative

- 20 CDR-grafted heavy chain variable region derived from murine monoclonal antibody TF8-5G9 and human antibody KOL is shown below. The CDR-grafted heavy chain is designated TF8HCDR1; murine residues were retained in the FR at residues 6, 17, 23, 24, 28, 29, 30, 48, 49,
- 25 68, 71, 73, 78, 88 and 91. CDRs are underlined.

10	20	30	35ab	50
QVQLVQSGGG	VVQPGRLRL	SCKASGFNIK	<u>DYYMH</u> --WVR	QAPGKGLEWIG
52abc	60	70	80 82abc	90
<u>LIDP</u> --ENGNTIYD	<u>PKFQGRFSIS</u>	ADTSK--NTAFL	QMDSLRPEDTAVY	
100	110			

- 30 YCARDNSYYF DYWGQTPVT VSS (SEQ ID NO:11)

-14-

The amino acid sequence of a representative  
 1 CDR-grafted light chain variable region derived from  
 murine monoclonal antibody TF8-5G9 and human antibody  
 REI is shown below. The CDR-grafted light chain is  
 designated TF8LCDR1; murine residues were retained in  
 5 the FR at residues 39, 41, 46 and 105. CDRs are  
 underlined.

	10	20	30	40	50
	DIQMTQSPSS	LSASVGDRVT	ITCKASQDIR	KYLNWYQOK	WKAPKTLIYY
10	60	70	80	90	100
	ATSLADGVPS	RFSGSGSGTD	YTFTISSLQP	EDIATYYCLO	HGESPYTFGQ
	GTKLEITR (SEQ ID NO:12)				

A CDR-grafted antibody containing variable  
 15 regions TF8HCDR1 and TF8LCDR1 has been demonstrated in  
 accordance with the present invention to be as effective  
 as murine monoclonal antibody TF8-5G9 in binding to  
 human tissue factor. It has been further discovered in  
 accordance with the present invention, by examination of  
 20 the molecular structure of murine monoclonal antibody  
 TF8-5G9, and by design, construction, and analysis of  
 CDR-grafted antibodies, that the FR regions can be  
 further humanized without the loss of antigen binding  
 activity. In particular, the FR region may retain the  
 25 human FR residue at residues 6, 17, 68, 73 and 78 of the  
 heavy chain, and residues 39, 41, 16 and 105 of the  
 light chain, with maintenance of antigen binding  
 activity.

In a most preferred embodiment, the heavy  
 30 chain variable region contains a FR derived from human  
 antibody KOL in which murine monoclonal antibody TF8-5G9

35



-15-

residues are retained at amino acids 23, 24, 28, 29, 30,  
 1 48, 49, 71, 88 and 91. The preferred heavy chain  
 variable region is designated TF8HCDR20 and has the  
 following sequence.

```

5           10           20           30   35ab           50
  QVQLVESGGG VVQPGRLRL SCKASGFNIK DYYMH--WVR QAPGKLEWIGL

52abc       60           70           80 82abc       90           100
  IDP--ENGNTIYD PKFQGRFTIS ADNSKNTLFL QMDSLRLPEDTAVY YCARDNSYYF

10           110
  DYWGQGTPVT VSS (SEQ ID NO:13)
  
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In a most preferred embodiment, the light  
 chain variable region contains a FR derived from human  
 15 antibody REI in which murine monoclonal antibody TF8-5G9  
 residues are retained at amino acids 39 and 105. The  
 preferred light chain variable region is designated  
 TF8LCDR20 and has the following sequence.

```

           10           20           30           40           50
  DIQMTQSPSS LSASVGRVT ITCKASQDIR KYLNWYQQKP GKAPKLLIYY
20           60           70           80           90           100
  ATSLADGVPS RFSGSGSGTD YTFTISLQP EDIATYYCLO HGESPYTFGQ
  GTKLEITR (SEQ ID NO:14)
  
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It is within the ken of the ordinarily skilled  
 25 artisan to make minor modifications of the foregoing  
 sequences, including amino acid substitutions, deletions  
 and insertions. Any such modifications are within the  
 scope of the present invention so long as the resulting  
 CDR-grafted antibody maintains the ability to bind to  
 30 and inhibit human tissue factor. The ordinarily skilled  
 artisan can assess the activity of the CDR-grafted

antibody with reference to the functional assays  
1 described hereinbelow.

The human constant region of the CDR-grafted  
antibodies of the present invention is selected to  
minimize effector function. The intended use of the  
5 CDR-grafted antibodies of the present invention is to  
block the coagulation cascade by inhibition of tissue  
factor, and thus antibody effector functions such as  
fixation of complement are not desirable. Antibodies  
with minimal effector functions include IgG2, IgG4, IgA,  
10 IgD and IgE. In a preferred embodiment of the present  
invention, the heavy chain constant region is the human  
IgG4 constant region, and the light chain constant  
region is the human IgG4 kappa constant region.

In that effector functions may not be  
15 desirable for therapeutic uses, the present invention  
further contemplates active fragments of the CDR-grafted  
antibodies, and in particular Fab fragments and F(ab')<sub>2</sub>  
fragments. Active fragments are those fragments capable  
of inhibiting human tissue factor. Fab fragments and  
20 F(ab')<sub>2</sub> fragments may be obtained by conventional means,  
for example by cleavage of the CDR-grafted antibodies of  
the invention with an appropriate proteolytic enzyme  
such as papain or pepsin, or by recombinant production.  
The active fragments maintain the antigen binding sites  
25 of the CDR-grafted antibodies and thus are similarly  
useful therapeutically.

The ability of the CDR-grafted antibodies  
designed and constructed as taught in accordance with  
the present invention to bind and inhibit human tissue  
30 factor can be assessed by functional assays. For  
example, in a rapid and convenient assay, expression

vectors containing nucleic acids encoding the CDR-  
1 grafted heavy and light chains can be co-transfected  
into suitable host cells and transiently expressed. The  
resulting antibodies can be assessed by standard assays  
for ability to bind human tissue factor, and for ability  
5 to compete for binding to tissue factor with the non-  
human antibody from which the CDRs are derived.

For example, transient expression of nucleic  
acids encoding the CDR-grafted heavy and light chains in  
COS cells provides a rapid and convenient system to test  
10 antibody gene expression and function. Nucleic acids  
encoding the CDR-grafted heavy and light chains,  
respectively, are cloned into a mammalian cell  
expression vector, for example pSG5, described by Green  
et al. (1988) Nucleic Acids Res. 16:369 and commercially  
15 available from Stratagene Cloning Systems, La Jolla, CA.  
The pSG5 expression vector provides unique restriction  
sites for the insertion of the heavy and light chain  
genes, and in vivo expression is under the control of  
the SV40 early promoter. Transcriptional termination is  
20 signaled by the SV40 polyadenylation signal sequence.

The pSG5-based expression vectors containing  
nucleic acids encoding the heavy and light chains are  
cotransfected into COS cells and cultured under  
conditions suitable for transient expression. Cell  
25 culture media is then harvested and examined for  
antibody expression, for example by an enzyme linked  
immunosorbent assay (ELISA), to determine that suitable  
levels of antibody have been produced. An ELISA may  
then be used to assess the ability of the CDR-grafted  
30 antibody to bind to human tissue factor. Human tissue  
factor is immobilized on a microtiter plate and the COS

cell supernatant containing the CDR-grafted antibody is  
1 added followed by an incubation at room temperature for  
about one hour. The plates are then washed with a  
suitable detergent-containing buffer such as phosphate  
buffered saline (PBS)/Tween, followed by the addition of  
5 the components of a suitable detection system. For  
example, horseradish peroxidase conjugated goat anti-  
human kappa chain polyclonal antibody is added, followed  
by washing, followed by addition of substrate for  
horseradish peroxidase, and detection. The CDR-grafted  
10 antibodies within the scope of the present invention are  
those which are capable of binding to human tissue  
factor to a degree comparable to the non-human antibody  
from which the CDRs are derived as determined by the  
foregoing assay.

15           The ability of the CDR-grafted antibodies to  
inhibit the activity of human tissue factor in vivo can  
be conveniently assessed by the following in vitro assay  
that mimics in vivo coagulation events. In response to  
vascular injury in vivo, tissue factor binds to factor  
20 VII and facilitates the conversion of factor VII to a  
serine protease (factor VIIa). The factor VIIa-tissue  
factor complex converts factor X to a serine protease  
(factor Xa). Factor Xa forms a complex with factor Va  
(from the intrinsic coagulation pathway), resulting in  
25 the conversion of prothrombin to thrombin, which in turn  
results in the conversion of fibrinogen to fibrin. In a  
convenient in vitro functional assay, tissue factor is  
incubated in the presence of factor VIIa and the CDR-  
grafted anti-tissue factor antibody produced in the  
30 transient expression system described above. Factor X  
is added and the reaction mixture is incubated, followed

by an assay for factor Xa activity utilizing a  
1 chromogenic substrate for factor Xa (Spectrozyme FXa,  
American Diagnostica, Inc., Greenwich, CT). The ability  
of the CDR-grafted antibody to inhibit factor X  
activation thus provides a measure of the ability of the  
5 CDR-grafted antibody to inhibit the activity of human  
tissue factor.

The CDR-grafted antibodies within the scope of  
the present invention are those which are capable of  
inhibiting human tissue factor to a degree comparable to  
10 the non-human antibody from which the CDRs are derived  
as determined by the foregoing assay. In one  
embodiment, the CDR-grafted antibody has at least 50% of  
the inhibitory activity of TF8-5G9 for human tissue  
factor. In a preferred embodiment, the CDR-grafted  
15 antibody has at least 70% of the inhibitory activity of  
TF8-5G9 for human tissue factor. In a more preferred  
embodiment, the CDR-grafted antibody has at least 80% of  
the inhibitory activity of TF8-5G9 for human tissue  
factor. In a most preferred embodiment, the CDR-grafted  
20 antibody has at least 90% of the inhibitory activity of  
TF8-5G9 for human tissue factor.

In another embodiment, the present invention  
provides a method of producing a CDR-grafted antibody  
capable of inhibiting human tissue factor. The method  
25 comprises constructing an expression vector containing a  
nucleic acid encoding the CDR-grafted antibody heavy  
chain and an expression vector containing a nucleic acid  
encoding the CDR-grafted antibody light chain,  
transfecting suitable host cells with the expression  
30 vectors, culturing the transfected host cells under  
conditions suitable for the expression of the heavy and

light chains, and recovering the CDR-grafted antibody.

- 1 Alternately, one expression vector containing nucleic acids encoding the heavy and light chains may be utilized.

- Standard molecular biological techniques, for  
5 example as disclosed by Sambrook et al. (1989),  
Molecular Cloning: A Laboratory Manual Cold Spring Harbor Press, Cold Spring Harbor, NY may be used to obtain nucleic acids encoding the heavy and light chains of the CDR-grafted antibodies of the present invention.  
10 A nucleic acid encoding the CDR-grafted variable domain may be constructed by isolating cDNA encoding the antibody to be humanized, e.g. murine monoclonal antibody TF8-5G9, by conventional cloning methodology from the hybridoma producing the antibody, or by  
15 polymerase chain reaction (PCR) amplification of the variable region genes, as described for example by Winter et al., followed by site-directed mutagenesis to substitute nucleotides encoding the desired human residues into the FR regions. Alternately, the cDNA  
20 encoding the human antibody can be isolated, followed by site-directed mutagenesis to substitute nucleotides encoding the desired murine residues into the CDRs.

- Nucleic acids encoding the CDR-grafted variable domain may also be synthesized by assembling  
25 synthetic oligonucleotides, for example utilizing DNA polymerase and DNA ligase. The resulting synthetic variable regions may then be amplified by PCR. Nucleic acids encoding CDR-grafted variable domains may also be constructed by PCR strand overlap methods that are known  
30 in the art and reviewed by Owens et al.

Accordingly, having determined the desired  
1 amino acid sequences of the CDR-grafted variable domains  
in accordance with the present invention, the ordinarily  
skilled artisan can obtain nucleic acids encoding the  
variable domains. Further, the skilled artisan is aware  
5 that due to the degeneracy of the genetic code, various  
nucleic acid sequences can be constructed that encode  
the CDR-grafted variable domains. All such nucleic acid  
sequence are contemplated by the present invention.

The nucleic acids encoding the CDR-grafted  
10 variable domains are linked to appropriate nucleic acids  
encoding the human antibody heavy or light chain  
constant region. Nucleic acid sequences encoding human  
heavy and light chain constant regions are known in the  
art. It is within the ken of the ordinarily skilled  
15 artisan to include sequences that facilitate  
transcription, translation and secretion, for example  
start codons, leader sequences, the Kozak consensus  
sequence (Kozak, 1987, J. Mol. Biol. 196:947) and the  
like, as well as restriction endonuclease sites to  
20 facilitate cloning into expression vectors.

The present invention thus further provides  
nucleic acids encoding the heavy and light chains of  
CDR-grafted antibodies capable of inhibiting human  
tissue factor wherein the CDRs are derived from a murine  
25 monoclonal antibody against tissue factor and the FR and  
C regions are derived from one or more human antibodies.

In accordance with the present invention,  
representative nucleic acids encoding CDR-grafted heavy  
and light chains were constructed. The CDR-grafted  
30 heavy chain comprises a variable region containing FR  
regions derived from human antibody KOL and CDRs derived

from murine monoclonal antibody TF8-5G9 and further  
1 comprises a constant region derived from the heavy chain  
of human IgG4. The CDR-grafted light chain comprises a  
variable region containing FR regions derived from human  
antibody REI and CDRs derived from murine monoclonal  
5 antibody TF8-5G9 and further comprises a constant region  
derived from human IgG4 kappa chain. Nucleic acids  
encoding the heavy and light chains were constructed by  
assembling the variable regions from synthetic  
nucleotides, amplifying the assembled variable regions  
10 by PCR, purifying the amplified nucleic acids, and  
ligating the nucleic acid encoding the variable region  
into a vector containing a nucleic acid encoding the  
appropriate human constant region.

The sequences of representative nucleic acids  
15 encoding CDR-grafted heavy and light chains are  
presented as nucleotides 1-2360 of SEQ ID NO:15 and  
nucleotides 1-759 of SEQ ID NO:20, respectively.

The nucleic acid sequence encoding a preferred  
heavy chain (nucleotides 1-2360 of SEQ ID NO:15) is  
20 designated the TF8HCDR20 gene. The nucleic acid  
sequence contains the following regions: 5' EcoRI  
restriction site (nucleotides 1-6); Kozak sequence  
(nucleotides 7-15); start codon and leader sequence  
(nucleotides 16-72); CDR-grafted variable region  
25 (nucleotides 73-423); human IgG4 CH1 domain (nucleotides  
424-717); human IgG4 intron 2 (nucleotides 718-1110);  
human IgG4 hinge (nucleotides 1111-1146); human IgG4  
intron 3 (nucleotides 1147-1267); human IgG4 CH2 domain  
(nucleotides 1268-1594); human IgG4 intron 4  
30 (nucleotides 1595-1691); human IgG4 CH3 domain  
(nucleotides 1692-2012); 3' untranslated region



(nucleotides 2013-2354); 3' BamHI end spliced to BclI  
1 site of expression vector (nucleotides 2355-2360).

The nucleic acid sequence encoding a preferred  
light chain gene (nucleotides 1-759 of SEQ ID NO:20) is  
designated the TF8LCDR3 gene. The nucleic acid sequence  
5 contains the following regions: 5' EcoRI restriction  
site (nucleotides 1-5); Kozak sequence (nucleotides 6-  
8); start codon and leader sequence (nucleotides 9-68);  
CDR-grafted variable region (nucleotides 69-392); human  
kappa constant region (nucleotides 393-710); 3'  
10 untranslated region (nucleotides 711-753); 3' BamHI end  
spliced to BclI site of expression vector (nucleotides  
754-759).

The foregoing preferred sequences can be  
modified by the ordinarily skilled artisan to take into  
15 account degeneracy of the genetic code, and to make  
additions, deletions, and conservative and  
nonconservative substitutions that result in a  
maintenance of the function of the nucleic acid, i.e.  
that it encodes a heavy or light chain of a CDR-grafted  
20 antibody capable of inhibiting human tissue factor.  
Restriction sites and sequences that facilitate  
transcription and translation may be altered or  
substituted as necessary depending upon the vector and  
host system chosen for expression.

25 Suitable expression vectors and hosts for  
production of the CDR-grafted antibodies of the present  
invention are known to the ordinarily skilled artisan.  
The expression vectors contain regulatory sequences,  
such as replicons and promoters, capable of directing  
30 replication and expression of heterologous nucleic acids  
sequences in a particular host cell. The vectors may

also contain selection genes, enhancers, signal  
1 sequences, ribosome binding sites, RNA splice sites,  
polyadenylation sites, transcriptional terminator  
sequences, and so on. The vectors may be constructed by  
conventional methods well-known in the art, or obtained  
5 from commercial sources. The expression vectors  
preferably have convenient restriction sites at which  
the nucleic acids encoding the antibody chains of the  
invention are inserted. Myeloma expression vectors in  
which antibody gene expression is driven by the human  
10 cytomegalovirus promoter-enhancer or are particularly  
preferred.

Expression vectors containing a nucleic acid  
encoding the CDR-grafted heavy chain under the control  
of a suitable promoter and expression vectors containing  
15 a nucleic acid encoding the CDR-grafted light chain  
under the control of a suitable promoter are  
cotransfected into a suitable host cell. In another  
embodiment, nucleic acids encoding both heavy and light  
chains are provided in a single vector for transfection  
20 of a suitable host cell.

Suitable host cells or cell lines for  
expression of the CDR-grafted antibodies of the present  
invention include bacterial cells, yeast cells, insect  
cells, and mammalian cells such as Chinese hamster ovary  
25 (CHO) cells, COS cells, fibroblast cells and myeloid  
cells. Mammalian cells are preferred. CHO, COS and  
myeloma cells are particularly preferred. Myeloma cells  
are preferred for establishing permanent CDR-grafted  
antibody producing cell lines. Expression of antibodies  
30 in myeloma cells, bacteria, and yeast is reviewed by

Sandhu (1992) Critical Reviews in Biotechnology 12:437.

1 Expression in mammalian cells is reviewed by Owen et al.

Transfection of host cells by the expression vectors containing nucleic acids encoding the CDR-grafted heavy and light chains can be accomplished by

5 methods well-known to one of ordinary skill in the art.

Such methods include, for example, calcium chloride transfection, calcium phosphate transfection,

lipofection and electroporation. Suitable culture

methods and conditions for the production of the CDR-

10 grafted antibodies are likewise well-known in the art.

The CDR-grafted antibodies can be purified by

conventional methods, including ammonium sulfate

precipitation, affinity chromatography, gel

electrophoresis, and the like. The ability of the CDR-

15 grafted antibodies to bind to and inhibit human tissue factor can be assessed by the in vitro assays described above.

The CDR-grafted antibodies of the present invention have a variety of utilities. For example, the

20 antibodies are capable of binding to human tissue factor and thus are useful in assays for human tissue factor from body fluid samples, purification of human tissue factor, and so on.

The CDR-grafted antibodies of the present

25 invention are capable of inhibiting human tissue factor.

Human tissue factor is well-known to be an essential element in the human coagulation cascade. The ability

of the antibodies of the present invention to disrupt

the coagulation cascade is demonstrated by in vitro

30 assays in which the antibodies prevent factor X

activation. Accordingly, the present antibodies are

useful in the attenuation of coagulation. The present  
1 invention thus provides a method of attenuation of  
coagulation comprising administering a therapeutically  
effective amount of CDR-grafted antibody capable of  
inhibiting human tissue factor to a patient in need of  
5 such attenuation.

Numerous thrombotic disorders are  
characterized by excessive or inappropriate coagulation  
and are effectively treated or prevented by  
administration of agents that interfere with the  
10 coagulation cascade. Accordingly, the present invention  
further provides a method of treatment or prevention of  
a thrombotic disorder comprising administering a  
therapeutically effective amount of a CDR-grafted  
antibody capable of inhibiting human tissue factor to a  
15 patient in need of such treatment or prevention. In a  
preferred embodiment, the thrombotic disorder is  
intravascular coagulation, arterial restenosis or  
arteriosclerosis. The antibodies of the invention may be  
used in combination with other antibodies or therapeutic  
20 agents.

A therapeutically effective amount of the  
antibodies of the present invention can be determined by  
the ordinarily skilled artisan with regard to the  
patient's condition, the condition being treated, the  
25 method of administration, and so on. A therapeutically  
effective amount is the dosage necessary to alleviate,  
eliminate, or prevent the thrombotic disorder as  
assessed by conventional parameters. For example, a  
therapeutically effective dose of a CDR-grafted antibody  
30 of the present invention may be from about 0.1 mg to  
about 20 mg per 70 kg of body weight. A preferred

dosage is about 1.0 mg to about 5 mg per 70 kg of body  
1 weight.

A patient in need of such treatment is a  
patient suffering from a disorder characterized by  
inappropriate or excessive coagulation, or a patient at  
5 risk of such a disorder. For example, anticoagulant  
therapy is useful to prevent postoperative venous  
thrombosis, and arterial restenosis following balloon  
angioplasty.

The CDR-grafted antibodies of the present  
10 invention are useful in the same manner as comparable  
therapeutic agents, and the dosage level is of the same  
order of magnitude as is generally employed with those  
comparable therapeutic agents. The present antibodies  
may be administered in combination with a  
15 pharmaceutically acceptable carrier by methods known to  
one of ordinary skill in the art.

Another embodiment of the present invention is  
directed to a pharmaceutical composition comprising a  
least one CDR-grafted antibody capable of inhibiting  
20 human tissue factor and further comprising a  
pharmaceutically acceptable carrier. As used herein,  
"pharmaceutically acceptable carrier" includes any and  
all solvents, dispersion media, coatings, antibacterial  
and antifungal agents, isotonic and absorption delaying  
25 agents, and the like. The use of such media and agents  
for pharmaceutically active substances is well-known in  
the art. Except insofar as any conventional media or  
agent is incompatible with the active ingredient, its  
use in the therapeutic compositions is contemplated.  
30 Supplementary active ingredients can also be  
incorporated into the compositions.

The antibodies can be administered by well-  
1 known routes including oral and parenteral, e.g.,  
intravenous, intramuscular, intranasal, intradermal,  
subcutaneous, and the like. Parenteral administration  
and particularly intravenous administration is  
5 preferred. Depending on the route of administration,  
the pharmaceutical composition may require protective  
coatings.

The pharmaceutical forms suitable for  
injectionable use include sterile aqueous solutions or  
10 dispersions and sterile powders for the extemporaneous  
preparation of sterile injectable solutions or  
dispersions. In all cases the ultimate solution form  
must be sterile and fluid. Typical carriers include a  
solvent or dispersion medium containing, for example,  
15 water buffered aqueous solutions (i.e., biocompatible  
buffers), ethanol, polyol such as glycerol, propylene  
glycol, polyethylene glycol, suitable mixtures thereof,  
surfactants or vegetable oils. The antibodies may be  
incorporated into liposomes for parenteral  
20 administration. Sterilization can be accomplished by an  
art-recognized techniques, including but not limited to,  
addition of antibacterial or antifungal agents, for  
example, paraben, chlorobutanol, phenol, sorbic acid or  
thimersal. Further, isotonic agents such as sugars or  
25 sodium chloride may be incorporated in the subject  
compositions.

Production of sterile injectable solutions  
containing the subject antibodies is accomplished by  
incorporating these antibodies in the required amount in  
30 the appropriate solvent with various ingredients  
enumerated above, as required, followed by

sterilization, preferably filter sterilization. To  
1 obtain a sterile powder, the above solutions are vacuum-  
dried or freeze-dried as necessary.

The following examples further illustrate the  
present invention.

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EXAMPLE 1

1                    Isolation and Sequencing of TF8-5G9  
                    Light Chain (LC) and Heavy Chain (HC)

                    Two DNA libraries were generated from oligo  
5 (dT)-primed TF8-5G9 hybridoma RNA utilizing standard  
molecular biology procedures as described by Sambrook et al.  
The cDNA was cloned into the Librarian II plasmid  
vector from Invitrogen (San Diego, CA), and the  
libraries were screened for cDNA clones encoding murine  
10 IgG HC and LC. A full-length cDNA clone for the heavy  
chain could not be isolated, despite the construction of  
two independent libraries. A random primed TF8-5G9 cDNA  
library was generated to obtain the missing 5' sequence  
of the heavy chain. Consequently, the heavy chain cDNA  
15 was in two pieces: a 5' clone of 390 nucleotides and a  
3' clone of 1392 nucleotides. The two HC clones overlap  
by 292 nucleotides.

                    The HC and LC clones were completely sequenced  
by the dideoxy chain termination method of Sanger et al.  
20 (1977) Proc. Natl. Acad. Sci. USA 74:5463. To verify  
the variable region sequence, sequence was obtained from  
PCR-amplified cDNA that had been synthesized from total  
TF8-5G9 hybridoma RNA. Total TF8-5G9 hybridoma RNA was  
isolated by the guanidinium thiocyanate method of  
25 Chrigwin et al. (1970) Biochemistry 18:5294. cDNA was  
synthesized using the Perkin Elmer (Norwalk, CT) GeneAmp  
RNA Polymerase Chain Reaction (PCR) kit with an oligo  
(dT) primer. Components of the same kit were used in  
the PCR to amplify the LC and HC variable regions using  
30 primers based on the sequence that had been obtained for  
the cDNA clones. The amplified variable region



fragments were gel-purified and sequenced according to  
1 the method of Tracy et al. (1991) BioTechniques 11:68 on  
a Model 373A Applied Biosystems, Inc. (Foster City, CA)  
automated fluorescent DNA sequencer. The sequence for  
TF8-5G9 LC and HC obtained from RNA amplification and  
5 the sequence obtained from the cDNA clones agreed. The  
TF8-5G9 HC variable region sequence with protein  
translation is shown in Figure 1 and SEQ ID NO:1, and  
that for the LC is shown in Figure 2 and SEQ ID NO:3.

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EXAMPLE 2

## 1      Chimeric LC and HC Expression Vector Construction

5                    In order to test the binding activity of the CDR-grafted anti-TF LC and HC individually, mouse-human chimeric TF8-5G9 LC and HC were constructed. This allowed the CDR-grafted LC to be tested for TF binding ability in combination with the chimeric HC, and the CDR-grafted HC to be tested in combination with the chimeric LC.

10                   Primers were designed to amplify the TF8-5G9 LC variable region using as template cDNA clones in the Librarian II vector. The 5' primer was designed with an EcoRI site while the 3' primer was designed with a NarI site. PCR was used to amplify the LC variable region, generating a 433 bp fragment with a 5'EcoRI end and 3'NarI end. The fragment included the signal sequence from the TF8-5G9 LC cDNA clone but incorporated a 2 base change in the arginine codon immediately following the ATG start codon. This change retained the arginine residue but made the sequence conform to the Kozak consensus sequence in order to potentially improve translation of the LC mRNA. The PCR amplified LC variable region fragment was digested with EcoRI and NarI restriction enzymes and purified by electrophoresis on a 2% Nusieve, 1% Seakem agarose gel (FMC Bio Products, Rockland, ME).

20                   The DNA was extracted from the gel slice and purified by the Geneclean (Bio 101, La Jolla, CA) procedure. The full-length chimeric TF8-5G9 LC gene was generated by cloning this DNA into the EcoRI and NarI sites of a pSP73 vector (Promega, Madison, WI) which

contains the human kappa constant region. The gene was  
1 isolated from the pSP73 vector by EcoRI digestion and  
subcloned into the EcoRI site of the pSG5 mammalian cell  
expression vector (Stratagene Cloning Systems, La Jolla,  
CA).

5           The chimeric TF8-5G9 HC gene was assembled in  
a manner similar to that of the chimeric LC. Since  
there was no full-length HC cDNA isolated from the  
Librarian II vector cDNA libraries, the HC variable  
region fragment that was generated by the PCR from total  
10 TF8-5G9 hybridoma cell RNA was used as the template.  
Primers which incorporated an EcoRI site at the 5' end  
and a SacI site at the 3' end were used in the PCR to  
generate a 430 bp fragment which contained the TF8-5G9  
HC Kozak sequence, start codon, signal sequence, and  
15 variable region. This fragment was digested with the  
restriction enzymes EcoRI and SacI, and gel-purified  
using the same procedure that was used with the chimeric  
LC construction.

          The full-length TF8-5G9 chimeric HC gene was  
20 constructed by cloning the variable region fragment into  
the EcoRI and SacI sites of the pSG5 expression vector  
containing the human IgG4 constant region.

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EXAMPLE 3

1                   Design and Construction of the  
                  CDR-Grafted Heavy and Light Chain Genes

                  The variable region domains of the CDR-grafted  
5   HC and LC genes were designed with an EcoRI overhang at  
          the 5' end followed by a Kozak sequence to improve  
          antibody expression. The leader sequences were derived  
          from the heavy and light chains of the murine monoclonal  
          antibody B72.3 (Whittle et al. (1987) Protein  
10 Engineering 1:499). The 3' end of the variable regions  
          were designed to have overhangs which allowed for  
          splicing to the appropriate human constant region DNA.

                  In the initially designed CDR-grafted TF8-5G9  
          heavy and light chains the CDRs were derived from murine  
15 TF8-5G9 sequence while the frameworks were derived  
          primarily from human antibody sequence. The human  
          antibody KOL (Schmidt et al.) was used for the heavy  
          chain frameworks, while the human antibody dimer (Epp et  
          al.) was used for the light chain frameworks.

20               Several criteria were used to select murine  
          framework residues in the design of the TF8-5G9 CDR-  
          grafted heavy and light chain variable regions.  
          Framework residues which, at a particular position, are  
          idiosyncratic to TF8-5G9 were retained as murine  
25 sequence with the assumption that they contributed to  
          its unique binding characteristics. TF8-5G9 murine  
          residues were also retained at framework positions where  
          they were in agreement with the human consensus sequence  
          but where the corresponding residues in KOL or REI were  
30 idiosyncratic. Residues that are part of antibody loop  
          canonical structures such as residue 71 (numbering

according to Kabat et al.) of the heavy and light chains  
1 were also retained as murine sequence. Framework  
residues that form loops such as residues 26-30 of the  
HC were kept as TF8-5G9 murine sequence at positions  
were the murine sequence differed from the human.  
5 Residues known to directly influence the conformation of  
CDRs, such as 48 and 49 immediately preceding CDR2 of  
the HC, were also retained as murine sequence.

The amino acid sequence of the variable region  
for the initially designed CDR-grafted TF8-5G9 HC,  
10 TF8HCDR1, is shown in SEQ ID NO:11. Murine residues  
were retained at framework positions 6, 17, 23, 24, 28,  
29, 30, 48, 49, 68, 71, 73, 78 88 and 91. The CDR-  
grafted HC variable region was attached to a human IgG4  
constant region.

15 The amino acid sequence of the variable region  
for the initially designed CDR-grafted TF8-5G9 LC,  
TF8LCDR1, is shown in SEQ ID NO:12. Murine residues  
were retained at framework positions 39, 41, 46 and 105.  
The CDR-grafted LC variable region was attached to a  
20 human kappa constant region.

The variable region for the CDR-grafted HC and  
LC described above were each assembled from 13 synthetic  
oligonucleotides which were synthesized by Research  
Genetics, Inc., Huntsville, AL. These oligonucleotides  
25 ranged in length from 42 to 80 bases, and encoded both  
variable region strands. When the 6 complementary  
oligonucleotide pairs were annealed, the overhangs  
generated were 17 to 24 bases in length. These  
oligonucleotide pairs were combined, annealed at their  
30 complementary overhangs, and ligated to give the final  
full length double-stranded variable regions.

The HC variable region oligonucleotides were  
1 assembled into a 452 bp fragment which contains a 5'  
EcoRI site and a 3' SacI site. The polymerase chain  
reaction was used to amplify this fragment. The  
resulting amplified DNA was purified on a 2% Nusieve, 1%  
5 Seakem agarose gel (FMC). The appropriate size band of  
DNA was excised and the DNA was recovered by the  
Geneclean (Bio 101) procedure. The fragment was then  
digested with EcoRI and SacI, and purified again by the  
Geneclean method. This HC variable region fragment with  
10 EcoRI and SacI ends was cloned into the EcoRI and SacI  
sites of the pSport-1 vector (GIBCO-BRL Life  
Technologies, Gaithersburg, MD). DNA from several  
clones was isolated and sequenced to verify proper  
variable region assembly. All clones had unexpected  
15 base changes. One clone with the fewest base changes  
(two mismatches at bases 133 and 140) was selected to be  
corrected by site-directed mutagenesis according to  
Kunkel (1985) Proc. Natl. Acad. Sci. USA 82:488.  
Briefly, CJ236 (ung-, dut-) competent cells (Invitrogen  
20 Corporation, San Diego, CA) were transformed with the  
pSport vector containing the CDR-grafted HC variable  
region with the two base mismatch. Single-stranded,  
uridine-incorporated DNA templates were purified from  
phage following M13 helper phage (Stratagene Cloning  
25 Systems) infection of the transformed cells.  
Mutagenesis oligos containing the desired base changes  
were synthesized on an Applied Biosystems Model 380B DNA  
synthesizer. The mutagenesis oligos were annealed to  
the template DNA, and T7 DNA Polymerase and T4 DNA  
30 Ligase (MutaGene InVitro Mutagenesis Kit, Bo-Rad  
Laboratories, Richmond, CA) were used to incorporate the

oligo into a newly synthesized DNA strand. DH5 $\alpha$   
1 competent cells (GIBCO-BRL Life Technologies) were  
transformed with the double-stranded DNA. The original  
uridine-incorporated strand is destroyed while the newly  
synthesized strand containing the mutagenesis oligo is  
5 replicated. Phagemid DNA was prepared from the  
resulting mutagenesis clones and the variable regions  
were sequence to identify the clones which had  
incorporated the desired changes. The corrected HC  
EcoRI/SacI variable region fragment was excised from the  
10 pSport vector, purified and ligated into the EcoRI/SacI  
sites of a pSG5 vector containing the human IgG4  
constant region. This resulted in the generation of a  
full-length humanized TF8-5G9 HC gene, TF8HCDR1, in the  
pSG5 COS cell expression vector. The vector was  
15 designated pSG5TF8HCDR1.

The CDR-grafted TF8-5G9 LC variable region was  
also amplified by the PCR from the assembled synthetic  
oligonucleotides into a 433 bp fragment which contained  
a 5' EcoRI site and a 3' NarI site. This fragment was  
20 purified as described above for the HC, digested with  
EcoRI and NarI and purified by the Geneclean procedure.  
This fragment was cloned into the EcoRI and NarI sites  
of a pSG5 vector which contains the human kappa constant  
region. This resulted in the generation of a full-  
25 length humanized TF8-5G9 LC gene, TF8LCDR1, in the pSG5  
COS cell expression vector. Seven clones were  
sequenced, and one was found to have the desired CDR-  
grafted LC sequence. The vector was designated  
pSQ5TF8LCDR1.

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EXAMPLE 4

1                   Expression of the CDR-Grafted  
                  Heavy and Light Chain Genes in COS Cells

                  The transient expression of antibody genes in  
5 COS-1 cells provides a rapid and convenient system to  
test antibody gene expression and function. COS-1 cells  
were obtained from the American Type Culture Collection  
(CRL 1650) and cultured in Dulbecco's Modified Eagle  
Medium (DMEM, from GIBCO BRL Life Technologies) with 10%  
10 fetal calf serum. The pSG5TF8HCDR1 expression factor  
was cotransfected into COS cells with the pSG5 chimeric  
LC expression vector using the DEAE-Dextran method  
followed by DMSO shock as described by Lopata et al.  
(1984) Nucleic Acids Res. 14:5707. After 4 days of  
15 culture, media was harvested from the wells and examined  
for antibody expression levels.

                  Antibody levels were determined by an ELISA-  
based assembly assay. Plates were coated with a goat  
anti-human Fc specific antibody. Various dilutions of  
20 the COS cell supernatant containing secreted antibody  
were added, incubated for one hour, and washed. A  
horseradish peroxidase-linked goat anti-human kappa  
chain antibody was added, incubated for one hour at room  
temperature, and washed. Substrate for the horseradish  
25 peroxidase was added for detection. Antibody levels in  
the COS cell media were found to be nearly undetectable  
for the TF8HCDR1 x chimeric LC. Upon closer examination  
of the TF8HCDR1 variable region sequence, it was found  
that an unexpected base change, which had occurred  
30 during the site-directed mutagenesis process described  
in Example 3, introduced a stop codon into framework 4



of the TF8HCDR1 gene. This substitution was corrected  
1 by site-directed mutagenesis as described above.  
Thorough sequencing of the variable region confirmed  
that the correction was made with no additional changes  
introduced. Upon transfection of this corrected  
5 TF8HCDR1 gene with the chimeric LC, reasonable  
expression levels were obtained.

COS cells which had been co-transfected with  
the CDR-grafted LC expression vector, pSGTF8LCDR1, and  
either the chimeric HC or TF8HCDR1, produced antibody at  
10 reasonable levels. Antibody levels in COS cell  
supernatants ranged from 0.5  $\mu$ g to 10.0  $\mu$ g per ml.

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EXAMPLE 51     **Binding of the CDR-Grafted TF8-5G9 to Tissue Factor**

          An ELISA was used to determine the ability of  
the CDR-grafted TF8-5G9 antibody, TF8HCDR1 x TF8LCDR1,  
5     to bind to tissue factor. Tissue factor was immobilized  
on a microtiter plate. The test COS cell supernatant,  
containing the CDR-grafted antibody, was added to the  
well, incubated for one hour at room temperature.  
Following three washes with PBS/Tween, a goat anti-human  
10    kappa chain polyclonal antibody conjugated to  
horseradish peroxidase was added, incubated for one hour  
at room temperature and washed. Substrate for the  
horseradish peroxidase was added for detection. The  
positive control was the TF8-5G9 chimeric antibody. The  
15    CDR-grafted TF8-5G9 antibody was able to bind to tissue  
factor to a degree comparable to the chimeric TF8-5G9  
antibody (Figure 3, solid symbols).

          The ability of the humanized antibody to  
compete with murine TF8-5G9 for binding to tissue factor  
20    was also examined. Varying amounts of COS cell  
supernatant containing the test CDR-grafted antibody and  
a fixed amount of murine TF8-5G9 were added  
simultaneously to wells coated with tissue factor.  
Binding was allowed to occur for one hour at room  
25    temperature. The wells were washed three times with  
PBS/Tween. A goat anti-human kappa chain antibody  
conjugated to horseradish peroxidase was added,  
incubated for one hour at room temperature and washed.  
Substrate for the horseradish peroxidase was added for  
30    detection. The positive antibody competed as well as

the chimeric antibody with murine TF8-5G9 for binding to  
1 TF.

These data indicate that the initially  
designed CDR-grafted antibody, TF8HCDR1 x TF8LCDR1, was  
approximately as active as the chimeric TF8-5G9 in  
5 binding to TF and competing with the murine antibody for  
binding to TF.

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EXAMPLE 6

1                   **Construction and Characterization**  
                  **of Additional CDR-Grafted Heavy Chains**

                  Upon examination of the molecular structure of  
5 murine TF8-5G9, framework residues at positions 27, 68,  
73 and 78 were found to lie on the antibody surface and  
had no discernible contact with the CDRs. These  
framework residues were of murine sequence in TF8HCDR1  
but were changed to the human KOL sequence in various  
10 combinations to generate a series of CDR-grafted heavy  
chains with framework residue variations. The changes  
were made by the process of site-directed mutagenesis as  
described in Example 3. Each CDR-grafted heavy chain  
version was expressed in COS cells in combination with  
15 the CDR-grafted LC, TF8LCDR1, and tested for its ability  
to bind TF and compete with murine TF8-5G9 for binding.  
Every version of the CDR-grafted heavy chain in  
combination with TF8LCDR1 was shown to bind TF with an  
affinity comparable to chimeric TF8-5G9. Every CDR-  
20 grafted HC in combination with TF8LCDR1 was able to  
compete with murine TF8-5G9 for binding to TF to a  
degree comparable to the chimeric antibody.

                  Changes in sequence from murine to human for  
HC framework positions 6, 7, 68, 73 and 78 did not  
25 adversely affect the antigen binding ability of the  
antibody. The CDR-grafted HC version which had human  
sequence at all of these positions, and thus was the  
most humanized HC, was TF8HCDR20.

                  The complete sequence of the TF8HCDR20 gene  
30 was determined. The DNA sequence is shown as a 2360 bp  
EcoRI/BamHI insert with protein translation in the

pEe6TF8HCDR20 expression vector in Figure 4 and SEQ ID  
1 NO:15.

The essential regions of the gene are as  
follows:

	Nucleotide #	Region
5	1-6	5' <u>EcoRI</u> restriction site
	7-15	Kozak sequence
	16-72	Start codon and leader sequence
	73-423	CDR-grafted variable region
	424-717	Human IgG4 CH1 domain
10	718-1110	Human IgG4 intron 2
	1111-1146	Human IgG4 hinge
	1147-1267	Human IgG4 intron 3
	1268-1594	Human IgG4 CH2 domain
	1595-1691	Human IgG4 intron 4
15	1692-2012	Human IgG4 CH3 domain
	2013-2354	3' untranslated region
	2355-2360	3' <u>BamHI</u> end spliced to <u>BclI</u> site of the expression vector

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EXAMPLE 7

1                   Construction and Characterization  
                  of Additional CDR-Grafted Light Chains

                  The initially designed CDR-grafted LC,  
5 TF8LCDR1, contained four framework residues from the  
murine TF8-5G9 sequence. At two of these positions, 39  
and 105, the human REI framework sequence is unique to  
REI; however, the murine TF8-5G9 LC sequence is in  
agreement with the human consensus sequence. The other  
10 two murine framework residues, trp41 and thr46, are  
unique to TF8-5G9. Several versions of the CDR-grafted  
LC were generated in which the sequence at these four  
positions were changed from the murine to the human REI  
in various combinations. These changes were made by  
15 site-directed mutagenesis. Each version of the CDR-  
grafted LC was expressed in COS cells in combination  
with the CDR-grafted HC, TF8HCDR20, and tested for  
ability to bind tissue factor and compete with murine  
TF8-5G9 for binding. Every version of the CDR-grafted  
20 LC, in combination with TF8HCDR20, was shown to bind TF  
with an affinity comparable to TF8-5G9. Also every CDR-  
grafted LC version, in combination with TF8HCDR20, was  
able to compete with murine TF8-5G9 for binding to TF in  
a manner comparable to the chimeric TF8-5G9 control.

25                   Changes in sequence from murine to human for  
LC framework positions 39, 41, 46 and 105 did not  
adversely effect the ability of the antibody to  
recognize antigen. The CDR-grafted LC of choice was  
TF8LCDR3, where murine TF8-5G9 sequence was used at  
30 positions 39 and 105 because these are in agreement with

the human consensus sequence. The preferred CDR-grafted  
1 TF8-5G9 antibody is TF8HCDR20 x TF8LCDR3.

The complete sequence of the TF8LCDR3 gene was  
determined and is shown as a 759 bp EcoRI-BamHI insert  
with protein translation in the pEel2TF8LCDR3 expression  
5 vector in Figure 5 and SEQ ID NO:17. The essential  
regions of the gene are as follows:

	Nucleotide #	Region
	1-5	5' <u>Eco</u> RI restriction site
	6-8	Kozak sequence
10	9-68	Start codon and leader sequence
	69-392	CDR-grafted variable region
	393-710	Human kappa constant region
	711-753	3' untranslated region
15	754-759	3' <u>Bam</u> HI end spliced to <u>Bcl</u> I site of the expression vector

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EXAMPLE 8

1        CDR-Grafted TF8-5G9 Antibody TF8HCDR20 x TF8LCDR3  
         Inhibits Human Tissue Factor

         The binding of the CDR-grafted TF8-5G9  
5 antibody, TF8HCDR20 x TF8LCDR3, to TF was assessed as  
described in Example 5 and was found to be comparable to  
that of the chimeric TF8-5G9 as illustrated in Figure 6.  
The ability of the CDR-grafted TF8-5G9 to compete with  
the murine antibody for binding to TF is comparable to  
10 that of the chimeric TF8-5G9 as shown in Figure 7.

         An in vitro assay was used to measure the  
level of inhibition of factor X activation by the CDR-  
grafted TF8-5G9 antibody. In this assay, TF forms an  
active proteolytic complex with factor VII. This  
15 complex then converts factor X to factor Xa by  
proteolysis. The activated Xa enzymatically cleaves a  
substrate, Spectrozyme FXa, which releases a chromogen.  
The level of chromogen, as detected by optical density,  
is an indication of factor X activation due to TF-factor  
20 VIIa activity.

         The following reaction mixtures were prepared  
in 12 x 75 mm borosilicate glass tubes.

         25  $\mu$ l TBS (50 mM Tris, pH 7.4, 150 mM NaCl)  
         15  $\mu$ l 20 mM  $\text{CaCl}_2$ /1% bovine serum albumin  
25 (BSA)  
         20  $\mu$ l human placental tissue factor solution  
         (prepared by reconstituting one vial of  
         Thromborel S, Curtin Matheson Scientific  
         #269-338 with 4.0 ml  $\text{dH}_2\text{O}$  and diluting  
30 1:10 in TBS)



30  $\mu$ l Factor VII (Enzyme Research Labs #HFVII  
1 1007 at 237.66 ng/ml in TBS)  
30  $\mu$ l TBS or TF8-5G9 or TF8MCDR20 x TF8LCDR3  
at 1.18  $\mu$ g/ml or as indicated in Fig. 8  
The reaction mixtures were incubated at 37°C  
5 for ten minutes before the addition of Factor X. (In  
some cases the reaction mixture was preincubated for  
five minutes before addition of Factor VII or antibody,  
followed by a ten minute incubation before addition of  
Factor X.) Thirty  $\mu$ l of Factor X solution (Enzyme  
10 Research Labs, DHFX 330, 247.38  $\mu$ g/ml TBS) was added and  
the mixture was incubated at 37°C for three minutes.  
Factor X activation was terminated by pipetting 40  $\mu$ g of  
reaction mixture into 160  $\mu$ l of stop buffer (50 mM Tris,  
pH 7.4, 100 mM EDTA, 150 mM NaCl) in 96 well microtiter  
15 plates. Each tube of reaction mixture was pipetted into  
three microtiter wells. Fifty  $\mu$ l of Spectrozyme FXa  
substrate (American Diagnostica #222, 1 $\mu$ M/ml TBS) was  
added to each well. OD<sub>405</sub> was read on a Molecular  
Devices kinetic plate reader with readings taken every  
20 twenty seconds for ten minutes. Factor X activity was  
recorded as mOD/minute, and enzyme velocities over the  
linear portion of the reaction curve were compared to  
determine inhibition of factor X activation by the anti-  
TF antibodies.

25 As shown in Figure 8, the CDR-grafted TF8-5G9  
antibody is approximately as effective as the murine  
TF8-5G9 in inhibiting factor X activation. This  
indicates that the CDR-grafted TF8-5G9 is functionally  
active.

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EXAMPLE 9

1                   **Construction of the CDR-Grafted Heavy  
and Light Chain Myeloma Expression Vectors**

For the purpose of establishing a permanent  
5 CDR-grafted antibody-producing cell line, the TF8HCDR20  
and TF8LCDR3 genes were subcloned into myeloma cell  
expression vectors. The heavy chain TF8HCDR20 was  
subcloned into the EcoRI and BclI sites of the pEe6hCMV-  
BglII myeloma expression vector described by Stephens et  
10 al. (1989) Nucleic Acids Res. 17:7110 to produce  
pEe6TF8HCDR20. The light chain TF8LCDR3 was subcloned  
into the EcoTI and BclI sites of the pEel2 myeloma  
expression vector to produce pEel2TF8LCDR3. The heavy  
and light chain expression vectors are illustrated in  
15 Figures 9 and 10, respectively. In both vectors  
antibody gene transcription was driven by the human  
cytomegalovirus (hCMV) promoter-enhancer, which lies  
directly 5' to the multiple cloning site. The  
polyadenylation signal sequence lies 3' to the multiple  
20 cloning site and signals the termination of  
transcription. Each vector contains the  $\beta$ -lactamase  
gene to allow for ampicillin selection in E. coli. The  
pEel2 vector contains a glutamine synthetase cDNA gene  
under the transcriptional control of the SV40 early  
25 promoter. Glutamine synthetase allows for myeloma cell  
transfectants to be selected in glutamine-free media.  
Myeloma cells are devoid of glutamine synthetase  
activity and are dependent on a supply of glutamine in  
the culture media. Cells which have been transfected  
30 with the pEel2 vector, containing the glutamine

synthetase gene, are able to synthesize glutamine from  
1 glutamate and can survive in the absence of glutamine.

The pEe6TF8HCDR20 expression vector is a 7073 bp  
plasmid whose DNA sequence is shown in Figure 4 and SEQ  
ID NO:15. The coding regions of the TF8HCDR20 gene are  
5 translated. The essential regions of this vector are  
described below:

- 10 1. Nucleotides #1-2360: The TF8HCDR20 CDR-grafted HC gene is described in Example 6. The HC gene was inserted as an EcoRI/BamHI fragment into the EcoRI/BclI sites of the pEe6hCMV-BglII vector.
- 15 2. Nucleotides #2361-2593: This region encodes the SV40 early gene polyadenylation signal (SV40 nucleotides 2770-2537), which acts as a transcriptional terminator. This fragment is flanked by a 5' BclI site and a 3' BamHI site. The 3' BamHI end of the heavy chain gene was spliced to the 5' BclI site of the polyadenylation signal, thus eliminating both sites.
- 20 3. Nucleotides #2594-3848: This region is a BamHI-BglI fragment from pBR328 (nucleotides 375-2422) but with a deletion between the SalI and AvaI sites (pBR328 nucleotides 651-1425) following the addition of a SalI linker to the AvaI site. This region contains the Col E1 bacterial origin of replication.
- 25 4. Nucleotides #3849-4327: This is a BglI-XmnI fragment site from the  $\beta$ -lactamase gene of pSP64 (Promega Corporation, Madison, WI). This gene provides ampicillin resistance to bacteria transformed with this vector.
- 30 5. Nucleotides #4328-4885: This is an XmnI-HindIII fragment of the ColE1 based plasmid pCT54 described by Emtage et al. (1983) Proc. Natl. Acad. Sci. USA

80:3671. The HindIII site was converted to a BglII site by the addition of a linker following the addition of the hCMV promoter described below.

6. Nucleotides #4886-7022: These nucleotides encode the Pst-1m fragment of human cytomeglovirus (hCMV) strain AD 169 described by Greenway *et al.* (1982) Gene 18:355 containing the region coding for the hCMV middle intermediate early promoter. This Pst-1m fragment was cloned into the HindIII site of pEe6hCMV by addition of oligonucleotides of the following sequence to either end of the fragment:

5' GTCACCGTCCTTGACACGA 3'

3' ACGTCAGTGGCAGGAAGTGTGCTTCGA 5'

The resulting 2100 bp fragment was inserted such that the promoter directed transcription towards the EcoRI site of pEe6hCMV. The oligonucleotide above served to recreate the complete 5' untranslated sequence of the hCMV-MIE gene the added irrelevant sequence at the very 5' end of the fragment. The HindIII site at the 5' end was subsequently converted to a BglII site by the addition of a further linker.

7. Nucleotides #7023-7073: The pSP64 polylinker with the BamHI and SaII sites removed.

The pEel2TF8LCDR3 expression vector is a 7864 bp plasmid whose DNA sequence is shown in Figure 5 and SEQ ID NO:17. The coding regions of the TF8LCDR3 gene are translated. The essential regions of this expression vector are described below:

1. Nucleotides #1-759: The TF8LCDR3 CDR-grafted LC gene is described in Example 7. The gene was inserted as an

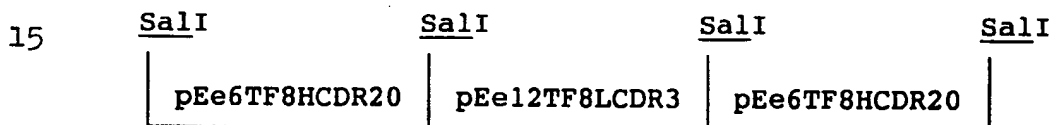
1            EcoRI/BamHI fragment into the EcoRI/BclII  
sites of the pEel2 expression vector.

2.        Nucleotides #760-3284: These regions of  
pEel2 are identical to the regions  
5        encoded by nucleotides 2361-4885 of the  
pEe6TF8HCDR20 vector described above  
(regions #2-5).

3.        Nucleotides #3285-5736: This region  
encodes the Chinese hamster ovary  
glutamine synthetase cDNA under the  
transcriptional control of the SV40 early  
promoter and followed by the SV40  
polyadenylation and splice signals from  
10       the pSV2.dhfr vector described by  
Subramani et al. (1981) Mol. Cell. Biol.  
1:854. The following describes the  
derivation of this region: A 1200 bp  
NaeI-PvuII fragment, containing a  
complete GS coding sequence, was excised  
from the Chinese hamster ovary cDNA clone  
15       λGS1.1 described by Hayward et al. (1986)  
Nucleic Acid Res. 14:999. After addition  
of a HindIII linker to the NaeI site and  
a BglII linker to the PvuII site (hence  
destroying the NaeI and PvuII sites), the  
1200 bp fragment was cloned in place of  
DHFR sequences in pSV2.dhfr between the  
20       HindIII and BglII sites to form pSV2.GS.  
The single remaining PvuII site in  
pSV2BamGS was converted to a BamHI site  
by addition of an oligonucleotide linker  
to form pSV2BamGS. An EcoRI site in the  
GS cDNA was destroyed by site directed  
mutagenesis without altering the amino  
acid sequence in pSV2BamGS and the  
25       HindIII site was destroyed by filling in  
with DNA polymerase I. The 2451 bp BamHI  
fragment from this plasmid, containing  
the complete SV40-GS hybrid transcription  
unit, was excised and inserted at the  
BglII site of pEe6hCMV-BglII site of  
pEe6hCMV-BglII such that transcription  
30       from the SV40 early promoter proceeds  
towards the hCMV promoter.

1        4.    Nucleotides #5737-7864: This region is  
          identical to the hCMV promoter and pSP64  
          polylinker encoded by nucleotides 4886-  
          7073 of the pEe6TF8HCDR20 vector  
          described above (regions 6 and 7).

         For the purpose of ensuring that both the  
 5    pEe6TF8HCDR20 and pEe12TF8LCDR3 vectors co-transfected  
      myeloma cells, the vectors were joined in linear  
      concatamers. Both the pEe6TF8HCDR20 and pEe12TF8LCDR3  
      vectors were digested at the unique SalI site. The SalI  
      linearized pEe6TF8HCDR20 vector was phosphatased at its  
 10    5' ends to prohibit ligation of two pEe6TF8HCDR20  
      vectors onto each other. This phosphatased HC vector  
      was ligated in a 2:1 molar ratio to the SalI linearized  
      pEe12TF8LCDR3. The resulting concatamers were most  
      likely of the following composition:



         This concatamerized DNA was extracted with phenol and  
      chloroform, and precipitated with ammonium acetate and  
 20    ethanol. The DNA precipitate was resuspended in  
      distilled water to a concentration of 1  $\mu\text{g}/\mu\text{L}$  and used  
      to transfect myeloma cells.

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EXAMPLE 10

## 1           Development of NSO Expression Cell Lines

Stably transformed cell lines expressing the humanized TF8-5G9 antibody were prepared by transfecting  
5 CDR-grafted heavy and light chain expression vectors into NSO mouse myeloma cells. Selection of transfected cells was carried out using the dominant selectable marker gene, glutamine synthetase (GS).

The NSO mouse myeloma cell line, obtained from  
10 Celltech, Ltd., is a subclone derived from NS-1 and does not express intracellular light chains. These cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with added glutamine and 10% fetal bovine serum (FBS). To prepare for transfection, the cells were  
15 harvested in mid-log phase of the growth cycle, centrifuged for 5 minutes, washed with phosphate buffered saline (PBS), centrifuged again, and the cell pellet was resuspended in 2.2 mL of PBS. The final cell concentration was  $2.18 \times 10^7$  mL. Cells were maintained  
20 on ice during the entire procedure.

The DNA to be transfected (pEel2TF8LCDR3 x pEe6TF8HCDR20) was prepared as a concatamer as described in Example 9. The DNA and NSO cells were added to a 0.4 cm BioRad Gene Pulser cuvette in the following order:

- 25           40  $\mu$ L (40  $\mu$ g) DNA concatamer  
            320  $\mu$ L double distilled water  
            40  $\mu$ L 10 x PBS  
            400  $\mu$ L NSO cells ( $8.72 \times 10^6$  cells)

Transfection was performed by electroporation  
30 following a protocol provided by Celltech, Ltd. In this procedure, the cells and DNA in PBS buffer were exposed

to a brief, high voltage pulse of electricity causing  
1 transient micropores to form on the cell membrane. DNA  
transfer takes place through these openings. To prepare  
for electroporation, the suspension of NSO cells and DNA  
was gently mixed and incubated on ice for 5 minutes.  
5 The cuvette was placed in a BioRad Gene Pulser and given  
2 consecutive electrical pulses at settings of 3  $\mu$ F  
(capacitance) and 1.5V (voltage). Following  
electroporation, the cuvette was returned to the ice for  
5 minutes. The suspension was then diluted in prewarmed  
10 growth medium and distributed into seven 96-well plates.  
Control plates containing cells electroporated without  
DNA were also prepared at the same time to measure the  
presence of spontaneous mutants. Plates were placed in  
a 37°C incubator with 5% CO<sub>2</sub>.  
15           Glutamine synthetase, encoded by the GS gene,  
is an enzyme that converts glutamate to glutamine. NSO  
cells require glutamine for growth due to inadequate  
levels of endogenous GS gene expression. In the DNA  
concatamer, this gene is located on the pEel2TF8LCDR3  
20 vector. Transfected cells which incorporate the GS gene  
become glutamine-independent. Cells not integrating the  
GS gene into their genome would remain glutamine-  
dependent and would not survive in glutamine-free  
medium. Approximately 18 hours post electroporation,  
25 all plates were fed with glutamine-free selection medium  
and returned to the incubator until viable colonies  
appeared.

          Approximately 3 weeks after transfection,  
distinct macroscopic colonies were observed. These were  
30 screened for expression of the intact humanized antibody  
using the assembly ELISA as described in Example 5.



Tissue culture supernatants from wells containing 1 colonies were screened at a 1:10 dilution. Positive wells showing activity greater than the 25 ng/mL standard were subcultured and expanded for further analysis.

5 For selection of high producers, antibody production was quantitated after a 96 hour growth period. Tissue culture flasks were seeded with  $2 \times 10^5$  cells/mL in 10 mL of selection medium and incubated at 37°C, 5% CO<sub>2</sub> for 96 hours. At the end of that time 10 period, an aliquot was taken to determine cell concentration and antibody titer. Evaluation of antibody production was calculated as µg/mL and pg/cell/96 hours. The highest producers from this transfection were:

15	<u>Cell Line</u>	<u>µg/mL</u>	<u>pg/cell/96 hour</u>
	2B1	26.3	24.3
	3E11	27.6	59.9
	4G6	30.2	41.9

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EXAMPLE 11

1            CDR Grafted Antibody TF8HCDR20 x TF8LCDR3  
             Inhibits Tissue Factor In Vivo

5            CDR grafted antibody TF8HCDR20 x TF8LCDR3 was  
compared to murine antibody TF8-5G9 for its ability to  
protect rats from experimentally induced disseminated  
intravascular coagulation (DIC). In the DIC model, rats  
are challenged with human thromboplastin (a crude tissue  
extract containing TF activity), resulting in fibrinogen  
10 consumption and death. Pretreatment of rats with anti-  
TF antibody was demonstrated to protect rats from  
fibrinogen consumption and death as follows.

Human thromboplastin was prepared as described  
in U.S. Patent 5,223,427. Saline control or 30  $\mu$ /ml of  
15 TF8-5G9 or CDR-grafted antibody was injected through the  
tail vein of rats, followed by injection of  
thromboplastin equivalent to 200 ng of recombinant TF.  
Clotting times were determined at T=0 and T=1 minute as  
a measure of fibrinogen concentration. Clotting times  
20 are proportional to fibrinogen concentration, with a 60  
second clotting time corresponding to an 80% reduction  
in fibrinogen concentration. Clotting times of greater  
than 60 seconds cannot be accurately measured and were  
recorded as 60 seconds.

25            Survivability and clotting times for three  
representative studies are shown below.

		<u>Survivors</u>		
Study		Controls	TF8-5G9	CDR-grafted Ab
30	1	0/8	5/8	6/8
	2	0/8	4/7	7/8
	3	0/8	8/8	3/7

<u>Clotting Times</u>						
<u>Controls</u>						
1	Study #1		Study #2		Study #3	
	<u>T=0</u>	<u>T=1</u>	<u>T=0</u>	<u>T=1</u>	<u>T=0</u>	<u>T=1</u>
5	16	>60	18	>60	19	>60
	16	>60	18	>60	21	>60
	16	>60	18	>60	18	>60
	17	>60	18	>60	19	>60
	15	>60	16	>60	18	54
	16	>60	18	>60	18	>60
	16	>60	17	>60	18	>60
	16	>60	17	>60	18	>60

<u>Clotting Times</u>						
<u>Murine TF8-5G9</u>						
10	Study #1		Study #2		Study #3	
	<u>T=0</u>	<u>T=1</u>	<u>T=0</u>	<u>T=1</u>	<u>T=0</u>	<u>T=1</u>
15	16	36	18	34	19	28
	15	41	18	36	18	29
	15	33	18	>60	19	29
	15	31	17	>60	18	29
	15	>60	18	50	18	28
	16	>60	17	34	19	40
	16	33	17	34	19	40
	16	33	18	31	19	34
20	16	>60			19	>60

<u>Clotting Times</u>						
<u>CDR-grafted TF8-5G9</u>						
25	Study #1		Study #2		Study #3	
	<u>T=0</u>	<u>T=1</u>	<u>T=0</u>	<u>T=1</u>	<u>T=0</u>	<u>T=1</u>
30	16	>60	17	>60	21	>60
	16	>60	17	33	18	34
	16	>60	18	32	17	>60
	22	37	18	>60	20	35
	16	32	17	32	17	58
	15	>60	18	31	18	33
	16	>60	17	31	18	31
	16	>60	16	32		

Twenty-three of the twenty-four control rats  
1 had clotting times of greater than 60 seconds indicating  
that virtually all untreated rats were consuming more  
than 80% of their fibrinogen. Both the CDR-grafted and  
murine antibody treated rats had similar clotting times  
5 at one minute of 44.5 and 40 seconds. Further, only six  
of the murine antibody treated rats and nine of the CDR-  
grafted antibody treated rats had clotting times in  
excess of 60 seconds. Accordingly, both the murine and  
CDR-grafted antibodies were able to neutralize TF and  
10 thus protect rats from fibrinogen consumption and death.

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SEQUENCE LISTING

1

## (1) GENERAL INFORMATION:

(i) APPLICANT: Joliffe, Linda K.  
Zivin, Robert A.  
5 Pulito, Virginia L.

(ii) TITLE OF INVENTION: CDR-GRAFTED ANTI-TISSUE FACTOR  
ANTIBODIES AND METHODS OF USE THEREOF

(iii) NUMBER OF SEQUENCES: 20

(iv) CORRESPONDENCE ADDRESS:  
10 (A) ADDRESSEE: Scully, Scott, Murphy & Presser  
(B) STREET: 400 Garden City Plaza  
(C) CITY: Garden City  
(D) STATE: New York  
(E) COUNTRY: United States  
(F) ZIP: 11530

(v) COMPUTER READABLE FORM:  
15 (A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:  
(A) APPLICATION NUMBER:  
(B) FILING DATE: 07-JUN-1995  
(C) CLASSIFICATION:

(viii)- ATTORNEY/AGENT INFORMATION:  
20 (A) NAME: DiGiglio, Frank S.  
(B) REGISTRATION NUMBER: 31,346  
(C) REFERENCE/DOCKET NUMBER: 9598

(ix) TELECOMMUNICATION INFORMATION:  
(A) TELEPHONE: (516) 742-4343  
(B) TELEFAX: (516) 742-4366  
(C) TELEX: 230 901 SANS UR

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## (2) INFORMATION FOR SEQ ID NO:1:

- 1 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1489 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: DNA (genomic)

- (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 11..1391

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

10	GGTCCTTACA ATG AAA TGC AGC TGG GTC ATC TTC TTC CTG ATG GCA GTG	49
	Met Lys Cys Ser Trp Val Ile Phe Phe Leu Met Ala Val	
	1 5 10	
	GTT ACA GGG GTC AAT TCA GAG ATT CAG CTG CAG CAG TCT GGG GCT GAG	97
	Val Thr Gly Val Asn Ser Glu Ile Gln Leu Gln Gln Ser Gly Ala Glu	
	15 20 25	
15	CTT GTG AGG CCA GGG GCC TTA GTC AAG TTG TCC TGC AAA GCT TCT GGC	145
	Leu Val Arg Pro Gly Ala Leu Val Lys Leu Ser Cys Lys Ala Ser Gly	
	30 35 40 45	
	TTC AAC ATT AAA GAC TAC TAT ATG CAC TGG GTG AAG CAG AGG CCT GAA	193
	Phe Asn Ile Lys Asp Tyr Tyr Met His Trp Val Lys Gln Arg Pro Glu	
	50 55 60	
	CAG GGC CTG GAG TGG ATT GGA TTG ATT GAT CCT GAG AAT GGT AAT ACT	241
	Gln Gly Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr	
	65 70 75	
20	ATA TAT GAC CCG AAG TTC CAG GGC AAG GCC AGT ATA ACA GCA GAC ACA	289
	Ile Tyr Asp Pro Lys Phe Gln Gly Lys Ala Ser Ile Thr Ala Asp Thr	
	80 85 90	
	TCC TCC AAC ACA GCC TAC CTG CAG CTC AGC AGC CTG ACA TCT GAG GAC	337
	Ser Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp	
	95 100 105	
25	ACT GCC GTC TAT TAC TGT GCT AGA GAT AAC TCG TAC TAC TTT GAC TAC	385
	Thr Ala Val Tyr Tyr Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr	
	110 115 120 125	

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1	TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC TCA GCC AAA ACG ACA CCC Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Pro	433
	130 135 140	
	CCA TCT GTC TAT CCA CTG GCC CCT GGA TCT GCT GCC CAA ACT AAC TCC Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser	481
	145 150 155	
5	ATG GTG ACC CTG GGA TGC CTG GTC AAG GGC TAT TTC CCT GAG CCA GTG Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val	529
	160 165 170	
	ACA GTG ACC TGG AAC TCT GGA TCC CTG TCC AGC GGT GTG CAC ACC TTC Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe	577
	175 180 185	
10	CCA GCT GTC CTG CAG TCT GAC CTC TAC ACT CTG AGC AGC TCA GTG ACT Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr	625
	190 195 200 205	
	GTG CCC TCC AGC ACC TGG CCC AGC GAG ACC GTC ACC TGC AAC GTT GCC Val Pro Ser Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala	673
	210 215 220	
	CAC CCG GCC AGC AGC ACC AAG GTG GAC AAG AAA ATT GTG CCC AGG GAT His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp	721
	225 230 235	
15	TGT GGT TGT AAG CCT TGC ATA TGT ACA GTC CCA GAA GTA TCA TCT GTC Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val	769
	240 245 250	
	TTC ATC TTC CCC CCA AAG CCC AAG GAT GTG CTC ACC ATT ACT CTG ACT Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr	817
	255 260 265	
20	CCT AAG GTC ACG TGT GTT GTG GTA GAC ATC AGC AAG GAT GAT CCC GAG Pro Lys Val Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu	865
	270 275 280 285	
	GTC CAG TTC AGC TGG TTT GTA GAT GAT GTG GAG GTG CAC ACA GCT CAG Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln	913
	290 295 300	
25	ACG CAA CCC CGG GAG GAG CAG TTC AAC AGC ACT TTC CGC TCA GTC AGT Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser	961
	305 310 315	

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-62-

1	GAA CTT CCC ATC ATG CAC CAG GAC TGG CTC AAT GGC AAG GAG TTC AAA Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys 320 325 330	1009
	TGC AGG GTC AAC AGT GCA GCT TTC CCT GCC CCC ATC GAG AAA ACC ATC Cys Arg Val Asn Ser Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile 335 340 345	1057
5	TCC AAA ACC AAA GGC AGA CCG AAG GCT CCA CAG GTG TAC ACC ATT CCA Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro 350 355 360 365	1105
	CCT CCC AAG GAG CAG ATG GCC AAG GAT AAA GTC AGT CTG AAC TGC ATG Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val Ser Leu Asn Cys Met 370 375 380	1153
10	ATA ACA GAC TTC TTC CCT GAA GAC ATT ACT GTG GAG TGG CAG TGG AAT Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn 385 390 395	1201
	GGG CAG CCA GCG GAG AAC TAC AAG AAC ACT CAG CCC ATC ATG GAC ACA Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr 400 405 410	1249
	GAT GGC TCT TAC TTC GTC TAC AGC AAG CTC AAT GTG CAG AAG AGC AAC Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn 415 420 425	1297
15	TGG GAG GCA GGA AAT ACT TTC ACC TGC TCT GTG TTA CAT GAG GGC CTG Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu 430 435 440 445	1345
	CAC AAC CAC CAT ACT GAG AAG AGC CTC TCC CAC TCT CCT GGT AAA T His Asn His His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys 450 455 460	1391
20	GATCCAGTG TCCTTGAGC CCTCTGGTCC TACAGGACTC TGACACCTAC CTCCACCCCT CCCTGTATAA ATAAAGCACC CAGCACTGCC TTGGACCC	1451 1489

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 460 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Lys Cys Ser Trp Val Ile Phe Phe Leu Met Ala Val Val Thr Gly  
 1 5 10 15  
 Val Asn Ser Glu Ile Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg  
 20 25 30  
 Pro Gly Ala Leu Val Lys Leu Ser Cys Lys Ala Ser Gly Phe Asn Ile  
 35 40 45  
 Lys Asp Tyr Tyr Met His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu  
 50 55 60  
 Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr Asp  
 65 70 75 80  
 Pro Lys Phe Gln Gly Lys Ala Ser Ile Thr Ala Asp Thr Ser Ser Asn  
 85 90 95  
 Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val  
 100 105 110  
 Tyr Tyr Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly Gln  
 115 120 125  
 Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Pro Pro Ser Val  
 130 135 140  
 Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser Met Val Thr  
 145 150 155 160  
 Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val Thr Val Thr  
 165 170 175  
 Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe Pro Ala Val  
 180 185 190  
 Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr Val Pro Ser  
 195 200 205  
 Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala His Pro Ala  
 210 215 220

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Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp Cys Gly Cys  
 225 230 235 240  
 1 Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val Phe Ile Phe  
 245 250 255  
 Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr Pro Lys Val  
 260 265 270  
 5 Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu Val Gln Phe  
 275 280 285  
 Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln Thr Gln Pro  
 290 295 300  
 Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser Glu Leu Pro  
 305 310 315 320  
 10 Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys Cys Arg Val  
 325 330 335  
 Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr  
 340 345 350  
 Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro Pro Pro Lys  
 355 360 365  
 15 Glu Gln Met Ala Lys Asp Lys Val Ser Leu Asn Cys Met Ile Thr Asp  
 370 375 380  
 Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn Gly Gln Pro  
 385 390 395 400  
 Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr Asp Gly Ser  
 405 410 415  
 20 Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn Trp Glu Ala  
 420 425 430  
 Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu His Asn His  
 435 440 445  
 His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys  
 450 455 460

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## (2) INFORMATION FOR SEQ ID NO:3:

- 1 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 937 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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- (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 5..706

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

10	GGAC ATG CGG GCC CCT GCT CAG TTT TTT GGG ATC TTG TTG CTC TGG TTT Met Arg Ala Pro Ala Gln Phe Phe Gly Ile Leu Leu Leu Trp Phe	49
	1 5 10 15	
	CCA GGT ATC AGA TGT GAC ATC AAG ATG ACC CAG TCT CCA TCC TCC ATG Pro Gly Ile Arg Cys Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met	97
	20 25 30	
15	TAT GCA TCG CTG GGA GAG AGA GTC ACT ATC ACT TGT AAG GCG AGT CAG Tyr Ala Ser Leu Gly Glu Arg Val Thr Ile Thr Cys Lys Ala Ser Gln	145
	35 40 45	
	GAC ATT AGA AAG TAT TTA AAC TGG TAC CAG CAG AAA CCA TGG AAA TCT Asp Ile Arg Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Trp Lys Ser	193
	50 55 60	
	CCT AAG ACC CTG ATC TAT TAT GCA ACA AGC TTG GCA GAT GGG GTC CCA Pro Lys Thr Leu Ile Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro	241
20	65 70 75	
	TCA AGA TTC AGT GGC AGT GGA TCT GGG CAA GAT TAT TCT CTA ACC ATC Ser Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile	289
	80 85 90 95	
	AGC AGC CTG GAG TCT GAC GAT ACA GCA ACT TAT TAC TGT CTA CAA CAT Ser Ser Leu Glu Ser Asp Asp Thr Ala Thr Tyr Tyr Cys Leu Gln His	337
	100 105 110	
25	GGT GAG AGC CCG TAC ACG TTC GGA GGG GGG ACC AAG CTG GAA ATA AAC Gly Glu Ser Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Asn	385
	115 120 125	

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1	AGG GCT GAT GCT GCA CCA ACT GTA TCC ATC TTC CCA CCA TCC AGT GAG Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu 130 135 140	433
	CAG TTA ACA TCT GGA GGT GCC TCA GTC GTG TGC TTC TTG AAC AAC TTC Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe 145 150 155	481
5	TAC CCC AAA GAC ATC AAT GTC AAG TGG AAG ATT GAT GGC AGT GAA CGA Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg 160 165 170 175	529
	CAA AAT GGC GTC CTG AAC AGT TGG ACT GAT CAG GAC AGC AAA GAC AGC Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser 180 185 190	577
10	ACC TAC AGC ATG AGC AGC ACC CTC ACG TTG ACC AAG GAC GAG TAT GAA Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu 195 200 205	625
	CGA CAT AAC AGC TAT ACC TGT GAG GCC ACT CAC AAG ACA TCA ACT TCA Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser 210 215 220	673
	CCC AAT GTC AAG AGC TTC AAC AAG AAT GAG TGT TAGAGACAAA GGCCTGAGA Pro Asn Val Lys Ser Phe Asn Lys Asn Glu Cys 225 230	726
15	CGCCACCACC AGCTCCCCAG CTCCATCCTA TCTTCCCTTC TAAGGTCTTG GAGGCTTCCC	786
	CACAAGCGAC CTACCACTGT TGCGGTGCTC CAAACCTCCT CCCCACCTCC TTCTCCTCCT	846
	CCTCCCTTTC CTTGGCTTTT ATCATGCTAA TATTGTCAGA AAATATTCAA TAAAGTGAGT	906
	CTTTGCACTT GAAAAAAAAA AAAAAAAAAA A	937

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## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 234 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

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## (ii) MOLECULE TYPE: protein

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

1 Met Arg Ala Pro Ala Gln Phe Phe Gly Ile Leu Leu Leu Trp Phe Pro  
    1                              5                              10                              15  
    Gly Ile Arg Cys Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met Tyr  
                               20                              25                              30  
    Ala Ser Leu Gly Glu Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp  
 5                              35                              40                              45  
    Ile Arg Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Trp Lys Ser Pro  
                               50                              55                              60  
    Lys Thr Leu Ile Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro Ser  
                               65                              70                              75                              80  
 10 Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile Ser  
                               85                              90                              95  
    Ser Leu Glu Ser Asp Asp Thr Ala Thr Tyr Tyr Cys Leu Gln His Gly  
                               100                              105                              110  
    Glu Ser Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Asn Arg  
                               115                              120                              125  
 15 Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln  
                               130                              135                              140  
    Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr  
                               145                              150                              155                              160  
    Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln  
                               165                              170                              175  
 20 Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr  
                               180                              185                              190  
    Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg  
                               195                              200                              205  
    His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro  
                               210                              215                              220  
 25 Asn Val Lys Ser Phe Asn Lys Asn Glu Cys  
                               225                              230

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## (2) INFORMATION FOR SEQ ID NO:5:

- 1 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 5 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- 5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
- Asp Asp Tyr Met His  
1 5

## 10 (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 17 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
- Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr Lys Pro Lys Phe Gln  
1 5 10 15  
Gly

20

## (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear
- 25 (ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

1      Asp Asn Ser Tyr Tyr Phe Asp Tyr  
      1                               5

(2) INFORMATION FOR SEQ ID NO:8:

5      (i) SEQUENCE CHARACTERISTICS:  
          (A) LENGTH: 11 amino acids  
          (B) TYPE: amino acid  
          (C) STRANDEDNESS: double  
          (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

10      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

      Lys Ala Ser Gln Asp Ile Arg Lys Tyr Leu Asn  
      1                               5                               10

(2) INFORMATION FOR SEQ ID NO:9:

15      (i) SEQUENCE CHARACTERISTICS:  
          (A) LENGTH: 7 amino acids  
          (B) TYPE: amino acid  
          (C) STRANDEDNESS: double  
          (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

20      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

      Tyr Ala Thr Ser Leu Ala Asp  
      1                               5

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## (2) INFORMATION FOR SEQ ID NO:10:

- 1 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 9 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- 5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
- Leu Gln His Gly Glu Ser Pro Tyr Thr  
 1 5

## 10 (2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 117 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
- Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg  
 1 5 10 15
- Leu Leu Arg Leu Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Tyr  
 20 25 30
- Tyr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
 35 40 45
- Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr Asp Pro Lys Phe  
 50 55 60
- Gln Gly Arg Phe Ser Ile Ser Ala Asp Thr Ser Lys Asn Thr Ala Phe  
 65 70 75 80

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-71-

1 Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys  
85 90 95  
Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Pro  
100 105 110  
Val Thr Val Ser Ser  
115

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## (2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 108 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

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## (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
1 5 10 15  
15 Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Ile Arg Lys Tyr  
20 25 30  
Leu Asn Trp Tyr Gln Gln Lys Pro Trp Lys Ala Pro Lys Thr Leu Ile  
35 40 45  
Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly  
50 55 60  
20 Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro  
65 70 75 80  
Glu Asp Ile Ala Thr Tyr Tyr Cys Leu Gln His Gly Glu Ser Pro Tyr  
85 90 95  
Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Thr Arg  
100 105

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## (2) INFORMATION FOR SEQ ID NO:13:

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- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 117 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

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- (ii) MOLECULE TYPE: peptide

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

10

Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val Gln Pro Gly Arg  
 1 5 10 15

Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Phe Asn Ile Lys Asp Tyr  
 20 25 30

Tyr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile  
 35 40 45

Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr Asp Pro Lys Phe  
 50 55 60

15

Gln Gly Arg Phe Thr Ile Ser Ala Asp Asn Ser Lys Asn Thr Leu Phe  
 65 70 75 80

Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95

Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly Gln Gly Thr Pro  
 100 105 110

Val Thr Val Ser Ser  
 115

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## (2) INFORMATION FOR SEQ ID NO:14:

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- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 108 amino acids  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly  
1 5 10 15  
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Ile Arg Lys Tyr  
5 20 25 30  
Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile  
35 40 45  
Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro Ser Arg Phe Ser Gly  
50 55 60  
Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr Ile Ser Ser Leu Gln Pro  
10 65 70 75 80  
Glu Asp Ile Ala Thr Tyr Tyr Cys Leu Gln His Gly Glu Ser Pro Tyr  
85 90 95  
Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Thr Arg  
100 105

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(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 7073 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: CDS  
(B) LOCATION: 61..717

(ix) FEATURE:

(A) NAME/KEY: CDS  
(B) LOCATION: 1111..1146

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(ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 1268..1594

(ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 1692..2012

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

	GAATTCGCCT CCACCATGGA ATGGAGCTGG GTCTTTCTCT TCTTCTTGTC AGTAACTACA	60
	GGT GTA CAC TCA CAA GTT CAG CTG GTG GAG TCT GGA GGA GGA GTA GTA	108
	Gly Val His Ser Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val	
	1 5 10 15	
	CAA CCT GGA AGG TCA CTG AGA CTG TCT TGT AAG GCT AGT GGA TTC AAT	156
10	Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Phe Asn	
	20 25 30	
	ATC AAG GAC TAT TAT ATG CAC TGG GTC AGA CAA GCT CCT GGA AAA GGA	204
	Ile Lys Asp Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly	
	35 40 45	
	CTC GAG TGG ATA GGT TTA ATT GAT CCT GAG AAT GGT AAC ACG ATA TAT	252
	Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr	
15	50 55 60	
	GAT CCC AAG TTC CAA GGA AGA TTC ATA ATT TCT GCA GAC AAC TCT AAG	300
	Asp Pro Lys Phe Gln Gly Arg Phe Ile Ile Ser Ala Asp Asn Ser Lys	
	65 70 75 80	
	AAT ACA CTG TTC CTG CAG ATG GAC TCA CTC AGA CCT GAG GAT ACA GCA	348
	Asn Thr Leu Phe Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala	
	85 90 95	
20	GTC TAC TTT TGT GCT AGA GAT AAC AGT TAT TAC TTC GAC TAC TGG GGC	396
	Val Tyr Phe Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly	
	100 105 110	
	CAA GGA ACA CCA GTC ACC GTG AGC TCA GCT TCC ACC AAG GGC CCA TCC	444
	Gln Gly Thr Pro Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser	
	115 120 125	
25	GTC TTC CCC CTG GCG CCC TGC TCC AGG AGC ACC TCC GAG AGC ACA GCC	492
	Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala	
	130 135 140	

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1	GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG	540
	Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val	
	145 150 155 160	
	TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT	588
	Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala	
	165 170 175	
5	GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG	636
	Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val	
	180 185 190	
	CCC TCC AGC AGC TTG GGC ACG AAG ACC TAC ACC TGC AAC GTA GAT CAC	684
	Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His	
	195 200 205	
10	AAG CCC AGC AAC ACC AAG GTG GAC AAG AGA GTT GGTGAGAGGC CAGCACAGGG	737
	Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val	
	210 215	
	CAGGGAGGGT GTCTGCTGGA AGCCAGGCTC AGCCCTCCTG CCTGGACGCA CCCC GGCTGT	797
	GCAGCCCCAG CCCAGGGCAG CAAGGCATGC CCCATCTGTC TCCTCACCCG GAGGCCTCTG	
	857	
	ACCACCCAC TCATGCTCAG GGAGAGGGTC TTCTGGATTT TTCCACCAGG CTCCGGGCAG	917
	CCACAGGCTG GATGCCCTA CCCCAGGCCC TGCGCATACA GGGG CAGGTG CTGCGCTCAG	
	977	
15	ACCTGCCAAG AGCCATATCC GGGAGGACCC TGCCCCTGAC CTAAGCCAC CCCAAAGGCC	1037
	AAACTCTCCA CTCCCTCAGC TCAGACACCT TCTCTCCTCC CAGATTCGAG TAACTCCCAA	
	1097	
	TCTTCTCTCT GCA GAG TCC AAA TAT GGT CCC CCA TGC CCA TCA TGC CCA	1146
	Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro	
	1 5 10	
20	GGTAAGCCAA CCCAGGCCTC GCCCTCCAGC TCAAGGCGGG ACAGGTGCCC TAGAGTAGCC	1206
	TGCATCCAGG GACAGGCCCC AGCCGGGTGC TGACGCATCC ACCTCCATCT CTTCCTCAGC	
	1266	
	A CCT GAG TTC CTG GGG GGA CCA TCA GTC TTC CTG TTC CCC CCA AAA	1312
	Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys	
	1 5 10 15	
25	CCC AAG GAC ACT CTC ATG ATC TCC CGG ACC CCT GAG GTC ACG TGC GTG	1360
	Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val	
	20 25 30	

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1	GTG GTG GAC GTG AGC CAG GAA GAC CCC GAG GTC CAG TTC AAC TGG TAC Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr 35 40 45	1408
	GTG GAT GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu 50 55 60	1456
5	CAG TTC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC ATG CAC Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Met His 65 70 75	1504
	CAG GAC TGG CTG AAC GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys 80 85 90 95	1552
10	GGC CTC CCG TCC TCC ATC GAG AAA ACC ATC TCC AAA GCC AAA Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys 100 105	1594
	GGTGGGACCC ACGGGGTGCG AGGGCCACAT GGACAGAGGT CAGCTCGGCC CACCCTCTGC	1654
	CCTGGGAGTG ACCGCTGTGC CAACCTCTGT CCCTACA GGG CAG CCC CGA GAG CCA Gly Gln Pro Arg Glu Pro 1 5	1709
15	CAG GTG TAC ACC CTG CCC CCA TCC CAG GAG GAG ATG ACC AAG AAC CAG Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln 10 15 20	1757
	GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAC CCC AGC GAC ATC GCC Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala 25 30 35	1805
20	GTG GAG TGG GAG AGT AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr 40 45 50	1853
	CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AGG CTA Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu 55 60 65 70	1901
25	ACC GTG GAC AAG AGC AGG TGG CAG GAG GGG AAT GTC TTC TCA GTC TCC Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Val Ser 75 80 85	1949

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1	GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACA CAG AAG AGC CTC TCC Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser 90 95 100	1997
	CTG TCT CTG GGT AAA TGAGTGCCAG GGCCGGCAAG CCCCCGCTCC CCGGGCTCTC Leu Ser Leu Gly Lys 105	2052
5	GGGGTCGCGC GAGGATGCTT GGCACGTACC CCGTCTACAT ACTTCCCAGG CACCCAGCAT GGAAATAAAG CACCCACCAC TGCCCTGGGC CCCTGTGAGA CTGTGATGGT TCTTTCCACG GGTCAGGCCG AGTCTGAGGC CTGAGTGACA TGAGGGAGGC AGAGCGGGTC CCACTGTCCC CACACTGGCC CAGGCTGTGC AGGTGTGCCT GGGCCACCTA GGGTGGGGCT CAGCCAGGGG CTGCCCTCGG CAGGGTGGGG GATTTGCCAG CGTGGCCCTC CCTCCAGCAG CAGGACTCTA	2112 2172 2232 2292 2352
10	GAGGATCATA ATCAGCCATA CCACATTTGT AGAGGTTTTA CTTGCTTTAA AAAACCTCCC ACACCTCCCC CTGAACCTGA AACATAAAAT GAATGCAATT GTTGTGTGTA ACTTGTTTAT TGCAGCTTAT AATGGTTACA AATAAAGCAA TAGCATCACA AATTTCACAA ATAAAGCATT TTTTTCACTG CATTCTAGTT GTGGTTTGTC CAACTCATC AATGTATCTT ATCATGTCTG	2412 2472 2532 2592
15	GATCCTCTAC GCCGGACGCA TCGTGCCCGG CATCACCGGC GCCACAGGTG CGGTTGCTGG CGCCTATATC GCCGACATCA CCGATGGGGA AGATCGGGCT CGCCACTTCG GGCTCATGAG CGCTTGTTTC GGC GTGGTA TGGTGGCAGG CCCGTGGCCG GGGGACTGTT GGGCGCCATC TCCTTG CATG CACCATT CCT TGCGGCGGCG GTGCTCAACG GCCTCAACCT ACTACTGGGC TGCTTCCTAA TGCAGGAGTC GCATAAGGGA GAGCGTCGAC CTCGGGCCGC GTTGTCTGGC	2652 2712 2772 2832 2892
20	TTTTTCCATA GGCTCCGCCC CCCTGACGAG CATCACAAAA ATCGACGCTC AAGTCAGAGG TGGCGAAACC CGACAGGACT ATAAAGATAC CAGGCGTTTC CCCCTGGAAG CTCCTCGTG CGCTCTCCTG TTCCGACCCT GCCGCTTACC GGATACCTGT CCGCCTTTCT CCCTTCGGGA AGCGTGGCGC TTTCTCAATG CTCACGCTGT AGGTATCTCA GTTCGGTGTA GGTCGTTCCG TCCAAGCTGG GCTGTGTGCA CGAACCCCC GTTCAGCCCG ACCGCTGCGC CTTATCCGGT	2952 3012 3072 3132 3192
25	AACTATCGTC TTGAGTCCAA CCCGGTAAGA CACGACTTAT CGCCACTGGC AGCAGCCACT	3252

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	GGTAACAGGA	TTAGCAGAGC	GAGGTATGTA	GGCGGTGCTA	CAGAGTTCTT	GAAGTGGTGG	3312
1	CCTAACTACG	GCTACACTAG	AAGGACAGTA	TTTGGTATCT	GCGCTCTGCT	GAAGCCAGTT	3372
	ACCTTCGGAA	AAAGAGTTGG	TAGCTCTTGA	TCCGGCAAAC	AAACCACCGC	TGGTAGCGGT	3432
	GGTTTTTTTG	TTTGCAAGCA	GCAGATTACG	CGCAGAAAAA	AAGGATCTCA	AGAAGATCCT	3492
	TTGATCTTTT	CTACGGGGTC	TGACGCTCAG	TGGAACGAAA	ACTCACGTTA	AGGGATTTTG	3552
5	GTCATGAGAT	TATCAAAAAG	GATCTTCACC	TAGATCCTTT	TAAATTAAAA	ATGAAGTTTT	3612
	AAATCAATCT	AAAGTATATA	TGAGTAAACT	TGGTCTGACA	GTTACCAATG	CTTAATCAGT	3672
	GAGGCACCTA	TCTCAGCGAT	CTGTCTATTT	CGTTCATCCA	TAGTTGCCTG	ACTCCCCGTC	3732
	GTGTAGATAA	CTACGATACG	GGAGGGCTTA	CCATCTGGCC	CCAGTGCTGC	AATGATACCG	3792
10	CGAGACCCAC	GCTCACCGGC	TCCAGATTTA	TCAGCAATAA	ACCAGCCAGC	CGGAAGGGCC	3852
	GAGCGCAGAA	GTGGTCCTGC	AACTTTATCC	GCCTCCATCC	AGTCTATTAA	TTGTTGCCGG	3912
	GAAGCTAGAG	TAAGTAGTTC	GCCAGTTAAT	AGTTTGCGCA	ACGTTGTTGC	CATTGCTACA	3972
	GGCATCGTGG	TGTCACGCTC	GTCGTTTGGT	ATGGCATCAT	TCAGCTCCGG	TTCCCAACGA	4032
	TCAAGGCGAG	TTACATGATC	CCCCATGTTG	TGCAAAAAG	CGGTTAGCTC	CTTCGGTCCT	4092
15	CCGATCGTTG	TCAGAAGTAA	GTTGGCCGCA	GTGTTATCAC	TCATGGTTAT	GGCAGCACTG	4152
	CATAATTCTC	TTACTGTCAT	GCCATCCGTA	AGATGCTTTT	CTGTGACTGG	TGAGTACTCA	4212
	ACCAAGTCAT	TCTGAGAATA	GTGTATGCGG	CGACCGAGTT	GCTCTTGCCC	GGCGTCAACA	4272
	CGGGATAATA	CCGCGCCACA	TAGCAGAACT	TTAAAAGTGC	TCATCATTGG	AAAACGTTCT	4332
20	TCGGGGCGAA	AACTCTCAAG	GATCTTACCG	CTGTTGAGAT	CCAGTTCGAT	GTAACCCACT	4392
	CGTGCACCCA	ACTGATCTTC	AGCATCTTTT	ACTTTCACCA	GCGTTTCTGG	GTGAGCAAAA	4452
	ACAGGAAGGC	AAAATGCCGC	AAAAAAGGGA	ATAAGGGCGA	CACGGAAATG	TTGAATACTC	4512
	ATACTCTTCC	TTTTTCAATA	TTATTGAAGC	ATTATCAGG	GTTATTGTCT	CATGAGCGGA	4572
	TACATATTTG	AATGTATTTA	GAAAAATAAA	CAAATAGGGG	TTCCGCGCAC	ATTCCCCGA	4632
25	AAAGTGCCAC	CTGACGTCTA	AGAAACCATT	ATTATCATGA	CATTAACTA	TAAAAATAGG	4692

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CGTATCACGA GGCCCTGATG GCTCTTTGCG GCACCCATCG TTCGTAATGT TCCGTGGCAC 4752  
1 CGACGACAAC CCTCAAGAGA AAATGTAATC AACTGGGCTC ACCTTCGGGT GGGCCTTTCT 4812  
GCGTTTATAA GGAGACACTT TATGTTTAAG AAGGTTGGTA AATTCCTTGC GGCTTTGGCA 4872  
GCCAAGCTAG AGATCTCTAG CTTCGTGTCA AGGACGGTGA CTGCAGTGAA TAATAAAATG 4932  
TGTGTTTGTG CGAAATACGC GTTTTGAGAT TTCTGTGCGC GACTAAATTC ATGTCGCGCG 4992  
5 ATAGTGGTGT TTATCGCCGA TAGAGATGGC GATATTGGAA AAATCGATAT TTGAAAATAT 5052  
GGCATATTGA AAATGTGCGC GATGTGAGTT TCTGTGTAAC TGATATCGCC ATTTTCCAA 5112  
AAGTGATTTT TGGGCATACG CGATATCTGG CGATAGCGCT TATATCGTTT ACGGGGGATG 5172  
GCGATAGACG ACTTTGGTGA CTTGGGCGAT TCTGTGTGTC GCAAATATCG CAGTTTCGAT 5232  
10 ATAGGTGACA GACGATATGA GGCTATATCG CCGATAGAGG CGACATCAAG CTGGCACATG 5292  
GCCAATGCAT ATCGATCTAT ACATTGAATC AATATTGGCC ATTAGCCATA TTATTCATTG 5352  
GTTATATAGC ATAAATCAAT ATTGGCTATT GGCCATTGCA TACGTTGTAT CCATATCATA 5412  
ATATGTACAT TTATATTGGC TCATGTCCAA CATTACCGCC ATGTTGACAT TGATTATTGA 5472  
CTAGTTATTA ATAGTAATCA ATTACGGGGT CATTAGTTCA TAGCCCATAT ATGGAGTTCC 5532  
15 GCGTTACATA ACTTACGGTA AATGGCCCGC CTGGCTGACC GCCCAACGAC CCCC GCCCAT 5592  
TGACGTCAAT AATGACGTAT GTTCCCATAG TAACGCCAAT AGGGACTTTC CATTGACGTC 5652  
AATGGGTGGA GTATTTACGG TAAACTGCCC ACTTGGCAGT ACATCAAGTG TATCATATGC 5712  
CAAGTACGCC CCCTATTGAC GTCAATGACG GTAAATGGCC CGCCTGGCAT TATGCCCAGT 5772  
20 ACATGACCTT ATGGGACTTT CCTACTTGGC AGTACATCTA CGTATTAGTC ATCGCTATTA 5832  
CCATGGTGAT GCGGTTTTGG CAGTACATCA ATGGGCGTGG ATAGCGGTTT GACTCACGGG 5892  
GATTTCCAAG TCTCCACCCC ATTGACGTCA ATGGGAGTTT GTTTTGGCAC CAAAATCAAC 5952  
GGGACTTTCC AAAATGTCGT AACAACTCCG CCCCATTGAC GCAAATGGGC GGTAGGCGTG 6012  
TACGGTGGGA GGTCTATATA AGCAGAGCTC GTTTAGTGAA CCGTCAGATC GCCTGGAGAC 6072  
25 GCCATCCACG CTGTTTTGAC CTCCATAGAA GACACCGGGA CCGATCCAGC CTCCGCGGCC 6132

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GGGAACGGTG CATTGGAACG CGGATTCCCC GTGCCAAGAG TGACGTAAGT ACCGCCTATA 6192
1 GAGTCTATAG GCCCACCCCC TTGGCTTCTT ATGCATGCTA TACTGTTTTT GGCTTGGGGT 6252
CTATACACCC CCGCTTCCTC ATGTTATAGG TGATGGTATA GCTTAGCCTA TAGGTGTGGG 6312
TTATTGACCA TTATTGACCA CTCCCTATT GGTGACGATA CTTTCCATTA CTAATCCATA 6372
ACATGGCTCT TTGCCACAAC TCTCTTTATT GGCTATATGC CAATACACTG TCCTTCAGAG 6432
5 ACTGACACGG ACTCTGTATT TTTACAGGAT GGGGTCTCAT TTATTATTTA CAAATTCACA 6492
TATACAACAC CACCGTCCCC AGTGCCCGCA GTTTTATTA AACATAACGT GGGATCTCCA 6552
CGCGAATCTC GGGTACGTGT TCCGGACATG GGCTCTTCTC CGGTAGCGGC GGAGCTTCTA 6612
CATCCGAGCC CTGCTCCCAT CCCTCCAGCG ACTCATGGTC GCTCGGCAGC TCCTTGCTCC 6672
10 TAACAGTGGA GGCCAGACTT AGGCACAGCA CGATGCCCAC CACCACCAGT GTGCCGCACA 6732
AGGCCGTGGC GGTAGGGTAT GTGTCTGAAA ATGAGCTCGG GGAGCGGGCT TGCACCGCTG 6792
ACGCATTTGG AAGACTTAAG GCAGCGGCAG AAGAAGATGC AGGCAGCTGA GTTGTTGTGT 6852
TCTGATAAGA GTCAGAGGTA ACTCCCGTTG CGGTGCTGTT AACGGTGGAG GGCAGTGTAG 6912
TCTGAGCAGT ACTCGTTGCT GCCGCGCGCG CCACCAGACA TAATAGCTGA CAGACTAACA 6972
15 GACTGTTTCT TTCCATGGGT CTTTCTGCA GTCACCGTCC TTGACACGAA GCTTGGGCTG 7032
CAGGTCGATC GACTCTAGAG GATCGATCCC CGGGCGAGCT C 7073

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## (2) INFORMATION FOR SEQ ID NO:16:

- 20 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 219 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

1 Gly Val His Ser Gln Val Gln Leu Val Glu Ser Gly Gly Gly Val Val  
    1                              5                              10                              15  
   Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala Ser Gly Phe Asn  
                               20                              25                              30  
 5 Ile Lys Asp Tyr Tyr Met His Trp Val Arg Gln Ala Pro Gly Lys Gly  
                               35                              40                              45  
   Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr Ile Tyr  
                               50                              55                              60  
   Asp Pro Lys Phe Gln Gly Arg Phe Ile Ile Ser Ala Asp Asn Ser Lys  
                               65                              70                              75                              80  
 10 Asn Thr Leu Phe Leu Gln Met Asp Ser Leu Arg Pro Glu Asp Thr Ala  
                               85                              90                              95  
   Val Tyr Phe Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr Trp Gly  
                               100                              105                              110  
   Gln Gly Thr Pro Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser  
                               115                              120                              125  
 15 Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala  
                               130                              135                              140  
   Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val  
                               145                              150                              155                              160  
   Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala  
                               165                              170                              175  
 20 Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val  
                               180                              185                              190  
   Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His  
                               195                              200                              205  
   Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val  
                               210                              215

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## (2) INFORMATION FOR SEQ ID NO:17:

- 1 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 12 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

- 5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro  
 1 5 10

## (2) INFORMATION FOR SEQ ID NO:18:

- 10 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 109 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

- 15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Pro Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro  
 1 5 10 15

Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val  
 20 25 30

- 20 Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val  
 35 40 45

Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln  
 50 55 60

Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Met His Gln  
 65 70 75 80

- 25 Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly  
 85 90 95

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1 Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys  
100 105

## (2) INFORMATION FOR SEQ ID NO:19:

## (i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 107 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

10 Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu  
1 5 10 15  
Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe  
20 25 30  
Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu  
35 40 45  
15 Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe  
50 55 60  
Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly  
65 70 75 80  
Asn Val Phe Ser Val Ser Val Met His Glu Ala Leu His Asn His Tyr  
85 90 95  
20 Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys  
100 105

## (2) INFORMATION FOR SEQ ID NO:20:

## (i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 7864 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: peptide

1 (ix) FEATURE:  
(A) NAME/KEY: CDS  
(B) LOCATION: 9..711

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

5	AATTCACCAT GGGTGTGCCA ACTCAGGTAT TAGGATTACT GCTGCTGTGG CTTACAGATG	60
	CAAGATGTGA TATCCAAATG ACACAATCTC CTCTTCTCT AAGTGCTTCT GTCGGAGATA	120
	GAGTAACAAT TACATGTAAG GCGAGTCAGG ACATTAGAAA GTATTTAAAC TGGTATCAGC	180
	AAAAACCTGG GAAGGCTCCT AAGCTACTGA TTTATTATGC AACAAAGTTG GCAGATGGAG	240
	TACCTTCTAG ATTTTCTGGT TCTGGCTCTG GAACAGACTA CACATTCACA ATTTCTTCTC	300
10	TCCAACCTGA GGACATTGCT ACATACTACT GCCTACAACA TGGTGAGAGT CCGTATACAT	360
	TTGGACAAGG AACAAAATA GAGATCACAA GAACTGTTGC GGCGCCGTCT GTCTTCATCT	420
	TCCCGCCATC TGATGAGCAG TTGAAATCTG GAACTGCCTC TGTGTGTGTC CTGCTGAATA	480
	ACTTCTATCC CAGAGAGGCC AAAGTACAGT GGAAGGTGGA TAACGCCCTC CAATCGGGTA	540
15	ACTCCCAGGA GAGTGTCA CA GAGCAGGACA GCAAGGACAG CACCTACAGC CTCAGCAGCA	600
	CCCTGACGCT GAGCAAAGCA GACTACGAGA AACACAAAGT CTACGCCTGC GAAGTCACCC	660
	ATCAGGGCCT GAGCTCGCCC GTCACAAAGA GCTTCAACAG GGGAGAGTGT TAGAGGGAGA	720
	AGTGCCCCCA CCTGCTCCTC AGTTCCAGCC TGGGGATCAT AATCAGCCAT ACCACATTTG	780
	TAGAGGTTTT ACTTGCTTTA AAAAACCTCC CACACCTCCC CCTGAACCTG AAACATAAAA	840
20	TGAATGCAAT TGTGTGTGTT AACTTGTTTA TTGCAGCTTA TAATGGTTAC AAATAAAGCA	900
	ATAGCATCAC AAATTCACA AATAAAGCAT TTTTTCCTACT GCATTCTAGT TGTGGTTTGT	960
	CCAAACTCAT CAATGTATCT TATCATGTCT GGATCCTCTA CGCCGGACGC ATCGTGCCCG	1020
	GCATCACCGG CGCCACAGGT GCGGTTGCTG GCGCCTATAT CGCCGACATC ACCGATGGGG	1080
25	AAGATCGGGC TCGCCACTTC GGGCTCATGA GCGCTTGTTT CGGCGTGGGT ATGGTGGCAG	1140

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	GGCCGTGGCC	GGGGGACTGT	TGGGCGCCAT	CTCCTTGCA	GCACCATTCC	TGCGGCGGC	1200
1	GGTGCTCAAC	GGCCTCAACC	TACTACTGGG	CTGCTTCCTA	ATGCAGGAGT	CGCATAAGGG	1260
	AGAGCGTCGA	CCTCGGGCCG	CGTTGCTGGC	GTTTTTCCAT	AGGCTCCGCC	CCCCTGACGA	1320
	GCATCACAAA	AATCGACGCT	CAAGTCAGAG	GTGGCGAAAC	CCGACAGGAC	TATAAAGATA	1380
	CCAGGCGTTT	CCCCCTGGAA	GCTCCCTCGT	GCGCTCTCCT	GTTCCGACCC	TGCCGCTTAC	1440
5	CGGATACCTG	TCCGCCTTTC	TCCCTTCGGG	AAGCGTGGCG	CTTTCTCAAT	GCTCACGCTG	1500
	TAGGTATCTC	AGTTCGGTGT	AGGTCGTTTC	CTCCAAGCTG	GGCTGTGTGC	ACGAACCCCC	1560
	CGTTCAGCCC	GACCGCTGCG	CCTTATCCGG	TAACTATCGT	CTTGAGTCCA	ACCCGGTAAG	1620
	ACACGACTTA	TCGCCACTGG	CAGCAGCCAC	TGGTAACAGG	ATTAGCAGAG	CGAGGTATGT	1680
10	AGGCGGTGCT	ACAGAGTTCT	TGAAGTGGTG	GCCTAACTAC	GGCTACACTA	GAAGGACAGT	1740
	ATTTGGTATC	TGCGCTCTGC	TGAAGCCAGT	TACCTTCGGA	AAAAGAGTTG	GTAGCTCTTG	1800
	ATCCGGCAAA	CAAACCACCG	CTGGTAGCGG	TGGTTTTTTT	GTTTGCAAGC	AGCAGATTAC	1860
	GCGCAGAAAA	AAAGGATCTC	AAGAAGATCC	TTTGATCTTT	TCTACGGGGT	CTGACGCTCA	1920
	GTGGAACGAA	AACTCACGTT	AAGGGATTTT	GGTCATGAGA	TTATCAAAAA	GGATCTTCAC	1980
15	CTAGATCCTT	TTAAATTAAA	AATGAAGTTT	TAAATCAATC	TAAAGTATAT	ATGAGTAAAC	2040
	TTGGTCTGAC	AGTTACCAAT	GCTTAATCAG	TGAGGCACCT	ATCTCAGCGA	TCTGTCTATT	2100
	TCGTTTCATC	ATAGTTGCCT	GAATCCCCGT	CGTGTAGATA	ACTACGATAC	GGGAGGGCTT	2160
	ACCATCTGGC	CCCAGTGCTG	CAATGATACC	GCGAGACCCA	CGCTCACCAG	CTCCAGATTT	2220
20	ATCAGCAATA	AACCAGCCAG	CCGGAAGGGC	CGAGCGCAGA	AGTGGTCCTG	CAACTTTATC	2280
	CGCCTCCATC	CAGTCTATTA	ATTGTTGCCG	GGAAGCTAGA	GTAAGTAGTT	CGCCAGTTAA	2340
	TAGTTTGCGC	AACGTTGTTG	CCATTGCTAC	AGGCATCGTG	GTGTCACGCT	CGTCGTTTGG	2400
	TATGGCTTCA	TTCAGCTCCG	GTTCCCAACG	ATCAAGGCGA	GTTACATGAT	CCCCCATGTT	2460
	GTGCAAAAAA	GCGGTTAGCT	CCTTCGGTCC	TCCGATCGTT	GTCAGAAGTA	AGTTGGCCGC	2520
25	AGTGTTATCA	CTCATGGTTA	TGGCAGCACT	GCATAATTCT	CTTACTGTCA	TGCCATCCGT	2580

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	AAGATGCTTT	TCTGTGACTG	GTGAGTACTC	AACCAAGTCA	TTCTGAGAAT	AGTGTATGCG	2640
1	GCGACCGAGT	TGCTCTTGCC	CGGCGTCAAC	ACGGGATAAT	ACCGCGCCAC	ATAGCAGAAC	2700
	TTTAAAAGTG	CTCATCATTG	GAAAACGTTT	TTCGGGGCGA	AAACTCTCAA	GGATCTTACC	2760
	GCTGTTGAGA	TCCAGTTCGA	TGTAACCCAC	TCGTGCACCC	AACTGATCTT	CAGCATCTTT	2820
	TACTTTCACC	AGCGTTTCTG	GGTGAGCAAA	AACAGGAAGG	CAAAATGCCG	CAAAAAAGGG	2880
5	AATAAGGGCG	ACACGGAAAT	GTTGAATACT	CATACTCTTC	CTTTTTCAAT	ATTATTGAAG	2940
	CATTTATCAG	GGTTATTGTC	TCATGAGCGG	ATACATATTT	GAATGTATTT	AGAAAAATAA	3000
	ACAAATAGGG	GTTCCGCGCA	CATTTCCCCG	AAAAGTGCCA	CCTGACGTCT	AAGAAACCAT	3060
	TATTATCATG	ACATTAACTT	ATAAAAATAG	GCGTATCACG	AGGCCCTGAT	GGCTCTTTGC	3120
10	GGCACCCATC	GTTTCGTAATG	TTCCGTGGCA	CCGAGGACAA	CCCTCAAGAG	AAAATGTAAT	3180
	CACACTGGCT	CACCTTCGGG	TGGGCCTTTC	TGCGTTTATA	AGGAGACACT	TTATGTTTAA	3240
	GAAGGTTGGT	AAATTCCTTG	CGGCTTTGGC	AGCCAAGCTA	GAGATCCGGC	TGTGGAATGT	3300
	GTGTCAGTTA	GGGTGTGGAA	AGTCCCCAGG	CTCCCCAGCA	GGCAGAAGTA	TGCAAAGCAT	3360
	GCATCTCAAT	TAGTCAGCAA	CCAGGCTCCC	CAGCAGGCAG	AAGTATGCAA	AGCATGCATC	3420
15	TCAATTAGTC	AGCAACCATA	GTCCCGCCCC	TAACTCCGCC	CATCCCGCCC	CTAACTCCGC	3480
	CCAGTTCCGC	CCATTCTCCG	CCCCATGGCT	GACTAATTTT	TTTTATTTAT	GCAGAGGCCG	3540
	AGGCCGCCTC	GGCCTCTGAG	CTATTCCAGA	AGTAGTGAGG	AGGCTTTTTT	GGAGGCCTAG	3600
	GCTTTTGCAA	AAAGCTAGCT	TGGGGCCACC	GCTCAGAGCA	CCTTCCACCA	TGGCCACCTC	3660
20	AGCAAGTTCC	CACTTGAACA	AAAACATCAA	GCAAATGTAC	TTGTGCCTGC	CCCAGGGTGA	3720
	GAAAGTCCAA	GCCATGTATA	TCTGGGTTGA	TGGTACTGGA	GAAGGACTGC	GCTGCAAAAC	3780
	CCGCACCCTG	GACTGTGAGC	CCAAGTGTGT	AGAAGAGTTA	CCTGAGTGGA	ATTTTGATGG	3840
	CTCTAGTACC	TTTCAGTCTG	AGGGCTCCAA	CAGTGACATG	TATCTCAGCC	CTGTTGCCAT	3900
	GTTTCGGGAC	CCCTTCCGCA	GAGATCCCAA	CAAGCTGGTG	TTCTGTGAAG	TTTTCAAGTA	3960
25	CAACCGGAAG	CCTGCAGAGA	CCAATTTAAG	GCACTCGTGT	AAACGGATAA	TGGACATGGT	4020

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GAGCAACCAG CACCCCTGGT TTGGAATGGA ACAGGAGTAT ACTCTGATGG GAACAGATGG 4080  
1 GCACCCTTTT GGTGGCCCTT CCAATGGCTT TCCTGGGCCC CAAGGTCCGT ATTACTGTGG 4140  
TGTGGGCGCA GACAAAGCCT ATGGCAGGGA TATCGTGGAG GCTCACTACC GCGCCTGCTT 4200  
GTATGCTGGG GTCAAGATTA CAGGAACAAA TGCTGAGGTC ATGCCTGCCC AGTGGGAACT 4260  
CCAAATAGGA CCCTGTGAAG GAATCCGCAT GGGAGATCAT CTCTGGGTGG CCCGTTTCAT 4320  
5 CTTNCATCGA GTATGTGAAG ACTTTGGGGT AATAGCAACC TTTGACCCCA AGCCCATTC 4380  
TGGGAACCTG AATGGTGCAG GCTGCCATAC CAACTTTAGC ACCAAGGCCA TGC GGAGGA 4440  
GAATGGTCTG AAGCACATCG AGGAGGCCAT CGAGAACTA AGCAAGCGGC ACCGGTACCA 4500  
CATTGAGCC TACGATCCCA AGGGGGGCCT GGACAATGCC CGTGGTCTGA CTGGGTTCCT 4560  
10 CGAAACGTCC AACATCAACG ACTTTTCTGC TGGTGTGCGC AATCGCAGTG CCAGCATCCG 4620  
CATTCCCCCG ACTGTGCGCC AGGAGAAGAA AGGTTACTTT GAAGACCGCG GCCCCTCTGC 4680  
CAATTGTGAC CCCTTTGCAG TGACAGAAGC CATCGTCCGC ACATGCCTTC TCAATGAGAC 4740  
TGGCCACGAG CCCTTCCAAT AAAAAACTA ATTAGACTTT GAGTGATCTT GAGCCTTTCC 4800  
TAGTTCATCC CACCCCGCCC CAGAGAGATC TTTGTGAAGG AACCTTACTT CTGTGGTGTG 4860  
15 ACATAATTGG ACAAATACC TACAGAGATT TAAAGCTCTA AGGTAAATAT AAAATTTTTA 4920  
AGTGATATAAT GTGTTAACT ACTGATTCTA ATTGTTTGTG TATTTTAGAT TCCAACCTAT 4980  
GGAAGTATG AATGGGAGCA GTGGTGAAT GCCTTTAATG AGGAAAACCT GTTTTGCTCA 5040  
GAAGAAATGC CATCTAGTGA TGATGAGGCT ACTGCTGACT CTCAACATTC TACTCCTCCA 5100  
20 AAAAGAAGA GAAAGGTAGA ACACCCAAG GACTTTCCTT CAGAATTGCT AAGTTTTTTG 5160  
AGTCATGCTG TGTTTAGTAA TAGAACTCTT GCTTGCTTTG CTATTTACAC CACAAAGGAA 5220  
AAAGCTGCAC TGCTATACAA GAAATTATG GAAAAATATT CTGTAACCTT TATAAGTAGG 5280  
CATAACAGTT ATAATCATAA CATACTGTTT TTTCTTACTC CACACAGGCA TAGAGTGTCT 5340  
GCTATTAATA ACTATGCTCA AAAATTGTGT ACCTTTAGCT TTTTAATTTG TAAAGGGGTT 5400  
25 AATAAGGAAT ATTTGATGTA TAGTGCCTAG ACTAGAGATC ATAATCAGCC ATACCACATT 5460

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TGTAGAGGTT TTA CTT C CTT TAAAAAACCT CCCACACCTC CCCCTGAACC TGAAACATAA 5520  
 1 AATGAATGCA ATTGTTGTTG TTA CTT TGT TATTGCAGCT TATAATGGTT ACAAATAAAG 5580  
 CAATAGCATC ACAAATTTCA CAAATAAAGC ATTTTTTTTCA CTGCATTCTA GTTGTGGTTT 5640  
 GTCCAACTC ATCAATGTAT CTTATCATGT CTGGATCTCT AGCTTCGTGT CAAGGACGGT 5700  
 GACTGCAGTG AATAATAAAA TGTGTGTTT TCCGAAATAC GCGTTTTGAG ATTTCTGTCTG 5760  
 5 CCTACTAAAT TCATGTCGCG CGATAGTGGT GTTTATCGCC GATAGAGATG GCGATATTGG 5820  
 AAAATCGAT ATTTGAAAAT ATGGCATATT GAAAATGTCG CCGATGTGAG TTTCTGTGTA 5880  
 ACTGATATCG CCATTTTCC AAAAGTGATT TTTGGGCATA CGCGATATCT GGCGATAGCG 5940  
 CTTATATCGT TTACGGGGGA TGGCGATAGA CGACTTTGGT GACTTGGGCG ATTCTGTGTG 6000  
 10 TCGCAAATAT CGCAGTTTCG ATATAGGTGA CAGACGATAT GAGGCTATAT CGCCGATAGA 6060  
 GGCGACATCA AGCTGGCACA TGGCCAATGC ATATCGATCT ATACATTGAA TCAATATTGG 6120  
 CCATTAGCCA TATTATTCAT TGGTTATATA GCATAAATCA ATATTGGCTA TTGGCCATTG 6180  
 CATACGTTGT ATCCATATCA TAATATGTAC ATTTATATTG GCTCATGTCC AACATTACCG 6240  
 CCATGTTGAC ATTGATTATT GACTAGTTAT TAATAGTAAT CAATTACGGG GTCATTAGTT 6300  
 15 CATAGCCCAT ATATGGAGTT CCGCGTTACA TAACTTACGG TAAATGGCCC GCCTGGCTGA 6360  
 CCGCCCAACG ACCCCCGCCC ATTGACGTCA ATAATGACGT ATGTTCCCAT AGTAACGCCA 6420  
 ATAGGGACTT TCCATTGACG TCAATGGGTG GAGTATTTAC GGTAACTGC CCACTTGGA 6480  
 GTACATCAAG TGTATCATAT GCCAAGTACG CCCCTATTG ACGTCAATGA CGGTAAATGG 6540  
 20 CCCGCCTGGC ATTATGCCCA GTACATGACC TTATGGGACT TTCCTACTTG GCAGTACATC 6600  
 TACGTATTAG TCATCGCTAT TACCATGGTG ATGCGGTTTT GGCAGTACAT CAATGGGCGT 6660  
 GGATAGCGGT TTGACTCACG GGGATTTCCA AGTCTCCACC CCATTGACGT CAATGGGAGT 6720  
 TTGTTTTGGC ACCAAAATCA ACGGGACTTT CCAAATGTC GTAACAATC CGCCCATTTG 6780  
 ACGCAAATGG GCGGTAGGCG TGTACGGTGG GAGGTCTATA TAAGCAGAGC TCGTTTAGTG 6840  
 25 AACCGTCAGA TCGCCTGGAG ACGCCATCCA CGCTGTTTTG ACCTCCATAG AAGACACCGG 6900

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GACCGATCCA GCCTCCGCGG CCGGGAACGG TGCATTGGAA CGCGGATTCC CCGTGCCAAG 6960  
1 AGTGACGTAA GTACCGCCTA TAGAGTCTAT AGGCCCACCC CTTGGGCTTC TTATGCATGC 7020  
TATACTGTTT TTGGCTTCGG GTCTATACAC CCCCCTTCC TCATGTTATA GGTGATGGTA 7080  
TAGCTTAGCC TATAGGTGTG GGTATTGAC CATTATTGAC CACTCCCCTA TTGGTGACGA 7140  
TACTTTCCAT TACTAATCCA TAACATGGCT CTTTGCCACA ACTCTCTTTA TTGGCTATAT 7200  
5 GCCAATACAC TGCCTTCAG AACTGACAC GGACTCTGTA TTTTACAGG ATGGGGTCTC 7260  
ATTTATTATT TACAAATTCA CATATACAA ACCACCGTCC CCAGTGCCCG CAGTTTTTAT 7320  
TAAACATAAC GTGGGATCTC CACGCGAATC TCGGGTACGT GTTCCGGACA TGGGCTCTTC 7380  
TCCGGTAGCG GCGGAGCTTC TACATCCGAG CCCTGCTCCC ATGCCTCCAG CGACTCATGG 7440  
10 TCGCTCGGCA TCTCCTTGCT CCTAACAGTG GAGGCCAGAC TTAGGCACAG CACGATGCCC 7500  
ACCACCACCA GTGTGCCGCA CAAGGCCGTG GCGGTAGGGT ATGTGTCTGA AAATGAGCTC 7560  
GGGGAGCGGG CTGCAACCGC TGACGCATTT GGAAGACTTA AGGCAGCGGC AGAAGAAGAT 7620  
GCAGGCAGCT GAGTTGTTGT GTTCTGATAA GAGTCAGAGG TAACTCCCGT TGCGGTGCTG 7680  
TTAACGGTGG AGGGCAGTGT AGTCTGAGCA GTACTCGTTG CTGCCGCGCG CGCCACCAGA 7740  
15 CATAATAGCT GACAGACTAA CAGACTGTTT CTTTCCATGG GTCTTTTCTG CAGTCACCGT 7800  
CCTTGACACG AAGCTTGGGC TGCAGGTCGA TCGACTCTAG AGGATCGATC CCCGGGCGAG 7860  
CTCG 7864

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WHAT IS CLAIMED IS:

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1. A CDR-grafted antibody capable of inhibiting human tissue factor wherein the complementarity determining regions (CDRs) are derived  
5 from a non-human monoclonal antibody against tissue factor and the framework (FR) and constant (C) regions are derived from one or more human antibodies.

2. The CDR-grafted antibody of Claim 1 wherein said non-human monoclonal antibody is a murine  
10 antibody.

3. The CDR-grafted antibody of Claim 2 wherein said murine antibody is TF8-5G9.

4. The CDR-grafted antibody of Claim 1 wherein said CDRs of the heavy chain have the amino acid  
15 sequences:

CDR1 DDYMH (SEQ ID NO:5)

CDR2 LIDPENGNTIYDPKFQG (SEQ ID NO:6)

CDR3 DNSYYFDY (SEQ ID NO:7)

and said CDRs of the light chain have the amino acid  
20 sequences:

CDR1 KASQDIRKYLN (SEQ ID NO:8)

CDR2 YATSLAD (SEQ ID NO:9)

CDR3 LQHGESPYT (SEQ ID NO:10).

5. The CDR-grafted antibody of Claim 1  
25 wherein the FR of the heavy chain is derived from the human antibody KOL.

6. The CDR-grafted antibody of Claim 1 wherein the FR of the light chain is derived from the human antibody REI.

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7. The CDR-grafted antibody of Claim 1  
1 wherein the heavy chain variable region has the amino  
acid sequence of SEQ ID NO:11.

8. The CDR-grafted antibody of Claim 1 or 7  
wherein the light chain variable region has the amino  
5 acid sequence of SEQ ID NO:12.

9. The CDR-grafted antibody of Claim 1  
wherein the heavy chain variable region has the amino  
acid sequence of SEQ ID NO:13.

10. The CDR-grafted antibody of Claim 1 or 9  
10 wherein the light chain variable region has the amino  
acid sequence of SEQ ID NO:14.

11. The CDR-grafted antibody of Claim 1  
wherein the heavy chain constant region is the human  
IgG4 constant region.

15 12. The CDR-grafted antibody of Claim 10  
wherein the heavy chain constant region is the human  
IgG4 constant region.

13. The CDR-grafted antibody of Claim 1  
wherein the light chain constant region is the human  
20 kappa constant region.

14. The CDR-grafted antibody of Claim 10  
wherein the light chain constant region is the human  
kappa constant region.

15. CDR-grafted monoclonal antibody TF8HCDR1  
25 x TF8LCDR1.

16. CDR-grafted monoclonal antibody TF8HCDR20  
x TF8LCDR3.

17. A fragment of the CDR-grafted antibody of  
Claim 1 wherein said fragment is capable of inhibiting  
30 human tissue factor.

18. The fragment of Claim 17 wherein said  
1 fragment is an Fab or F(ab')<sub>2</sub> fragment.

19. A method of making the CDR-grafted  
antibody of Claim 1 comprising cotransfecting a host  
cell with an expression vector comprising a nucleic acid  
5 encoding the CDR-grafted antibody heavy chain and an  
expression vector comprising a nucleic acid encoding the  
CDR-grafted antibody light chain; culturing the  
transfected host cell; and recovering said CDR-grafted  
antibody.

10 20. A method of making the CDR-grafted  
antibody of Claim 1 comprising transfecting a host cell  
with an expression vector comprising a nucleic acid  
encoding the CDR-grafted antibody heavy chain and a  
nucleic acid encoding the CDR-grafted antibody light  
15 chain; culturing the transfected host cell; and  
recovering said CDR-grafted antibody.

21. The method of Claim 18 or 19 wherein said  
nucleic acid encoding the CDR-grafted antibody heavy  
chain has the sequence of nucleotides 1-2360 of SEQ ID  
20 NO:15.

22. The method of Claim 18 or 19 wherein said  
nucleic acid encoding the CDR-grafted light chain has  
the sequence of nucleotides 1-759 of SEQ ID NO:17.

23. The method of Claim 19 or 20 wherein said  
25 host cell is a bacterial cell, yeast cell, insect cell  
or mammalian cell.

24. The method of Claim 23 wherein said  
mammalian cell is a CHO cell, COS cell or myeloma cell.

25. The method of Claim 19 wherein said  
30 expression vector comprising a nucleic acid encoding the  
CDR-grafted antibody heavy chain is pEe6TF8HCDR20.

26. The method of Claim 19 wherein said  
1 expression vector comprising a nucleic acid encoding the  
CDR-grafted antibody light chain is pEel2TF8LCDR3.

27. A nucleic acid encoding the heavy chain  
of the CDR-grafted antibody of Claim 1.

5 28. A nucleic acid encoding the light chain  
of the CDR-grafted antibody of Claim 1.

29. The nucleic acid of Claim 27 having the  
sequence of nucleotides 1-2360 of SEQ ID NO:15.

30. The nucleic acid of Claim 28 having the  
10 sequence of nucleotides 1-759 of SEQ ID NO:17.

31. A method of attenuation of coagulation  
comprising administering a therapeutically effective  
amount of a CDR-grafted antibody capable of inhibiting  
human tissue factor to a patient in need of said  
15 attenuation.

32. The method of Claim 31 wherein said CDR-  
grafted antibody is TF8HCDR20 x TF84CDR3.

33. A method of treatment or prevention of  
thrombotic disorder comprising administering a  
20 therapeutically effective amount of a CDR-grafted  
antibody capable of inhibiting human tissue factor to a  
patient in need of said treatment or prevention.

34. The method of Claim 33 wherein said  
thrombotic disorder is intravascular coagulation,  
25 arterial restenosis or arteriosclerosis.

35. The method of Claim 33 or 34 wherein said  
CDR-grafted antibody is TF8HCDR20 x TF8LCDR3.

36. A pharmaceutical composition comprising  
at least one CDR-grafted antibody capable of inhibiting  
30 human tissue factor and a pharmaceutically acceptable  
carrier.

37. The pharmaceutical composition of Claim  
1 36 wherein said CDR-grafted antibody is TF8HCDR20 x  
TF8LCDR3.

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Sequence of the murine TF8-5G9 heavy chain cDNA with protein translation. The essential regions of the cDNA are as follows:

<b>FIG. 1 A</b>	<u>Nucleotides</u>	<u>Region</u>
	1-10	5' untranslated region.
	11-67	Start codon and leader sequence.
	68-418	Variable region.
	419-1390	Murine IgG1 constant region.
	1391-1489	3' untranslated region.

Sequence Range: 1 to 1489

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      10      20      30      40
      *      *      *      *
GGT CCT TAC A ATG AAA TGC AGC TGG GTC ATC TTC TTC CTG ATG GCA GTG
CCA GGA ATG T TAC TTT ACG TCG ACC CAG TAG AAG AAG GAC TAC CGT CAC
      Met Lys Cys Ser Trp Val Ile Phe Phe Leu Met Ala Val>

50      60      70      80      90
      *      *      *      *      *
GTT ACA GGG GTC AAT TCA GAG ATT CAG CTG CAG CAG TCT GGG GCT GAG
CAA TGT CCC CAG TTA AGT CTC TAA GTC GAC GTC GTC AGA CCC CGA CTC
Val Thr Gly Val Asn Ser Glu Ile Gln Leu Gln Gln Ser Gly Ala Glu>

100     110     120     130     140
      *      *      *      *      *
CTT GTG AGG CCA GGG GCC TTA GTC AAG TTG TCC TGC AAA GCT TCT GGC
GAA CAC TCC GGT CCC CGG AAT CAG TTC AAC AGG ACG TTT CGA AGA CCG
Leu Val Arg Pro Gly Ala Leu Val Lys Leu Ser Cys Lys Ala Ser Gly>

150     160     170     180     190
      *      *      *      *      *
TTC AAC ATT AAA GAC TAC TAT ATG CAC TCG GTG AAG CAG AGG CCT GAA
AAG TTG TAA TTT CTG ATG ATA TAC GTG ACC CAC TTC GTC TCC GGA CTT
Phe Asn Ile Lys Asp Tyr Tyr Met His Trp Val Lys Gln Arg Pro Glu>

200     210     220     230     240
      *      *      *      *      *
CAG GGC CTC GAG TCG ATT GGA TTG ATT GAT CCT GAG AAT GGT AAT ACT
GTC CCG GAC CTC ACC TAA CCT AAC TAA CTA GGA CTC TTA CCA TTA TGA
Gln Gly Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly Asn Thr>

250     260     270     280
      *      *      *      *
ATA TAT GAC CCG AAG TTC CAG GGC AAG GCC AGT ATA ACA GCA GAC ACA
TAT ATA CTG GGC TTC AAG GTC CCG TTC CCG TCA TAT TGT CGT CTG TGT
Ile Tyr Asp Pro Lys Phe Gln Gly Lys Ala Ser Ile Thr Ala Asp Thr>

290     300     310     320     330
      *      *      *      *      *
TCC TCC AAC ACA GCC TAC CTG CAG CTC AGC AGC CTG ACA TCT GAG GAC
AGC AGG TTG TGT CCG ATG GAC GTC GAG TCG TCG GAC TGT AGA CTC CTG
Ser Ser Asn Thr Ala Tyr Leu Gln Leu Ser Ser Leu Thr Ser Glu Asp>

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**FIG. 1 B**

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340          350          360          370          380
*          *          *          *          *
ACT GCC GTC TAT TAC TGT GCT AGA GAT AAC TCG TAC TAC TTT GAC TAC
TGA CCG CAG ATA ATG ACA CGA TCT CTA TTG AGC ATG ATG AAA CTG ATG
Thr Ala Val Tyr Tyr Cys Ala Arg Asp Asn Ser Tyr Tyr Phe Asp Tyr>

390          400          410          420          430
*          *          *          *          *
TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC TCA GCC AAA ACG ACA CCC
ACC CCG GTT CCG TGG TGA GAG TGT CAG AGG AGT CCG TTT TGC TGT GCG
Trp Gly Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Lys Thr Thr Pro>

440          450          460          470          480
*          *          *          *          *
CCA TCT GTC TAT CCA CTG GCC CCT GGA TCT GCT GCC CAA ACT AAC TCC
GGT AGA CAG ATA GGT GAC CCG GGA CCT AGA CGA CCG GTT TGA TTG AGG
Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn Ser>

490          500          510          520
*          *          *          *
ATG GTG ACC CTG GGA TGC CTG GTC AAG GGC TAT TTC CCT CAG CCA GTG
TAC CAC TGG GAC CCT ACG GAC CAG TTC CCG ATA AAG GGA CTC GGT CAC
Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro Val>

530          540          550          560          570
*          *          *          *          *
ACA GTG ACC TGG AAC TCT GGA TCC CTG TCC AGC GGT GTG CAC ACC TTC
TGT CAC TGG ACC TTG AGA CCT AGG GAC AGG TCG CCA CAC GTG TGG AAG
Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr Phe>

580          590          600          610          620
*          *          *          *          *
CCA GCT GTC CTG CAG TCT GAC CTC TAC ACT CTG AGC AGC TCA GTG ACT
GGT CGA CAG GAC GTC AGA CTG GAG ATG TGA GAC TCG TCG AGT CAC TGA
Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val Thr>

630          640          650          660          670
*          *          *          *          *
GTG CCC TCC AGC ACC TGG CCC AGC GAG ACC GTC ACC TGC AAC GTT GCC
CAC GGG AGG TCG TCG ACC GCG TCG CTC TGG CAG TCG ACG TTG CAA CCG
Val Pro Ser Ser Thr Trp Pro Ser Glu Thr Val Thr Cys Asn Val Ala>

680          690          700          710          720
*          *          *          *          *
CAC CCG GCC AGC AGC ACC AAG GTG GAC AAG AAA ATT GTG CCC AGG GAT
GTG GCG CCG TCG TCG TGG TTC CAC CTG TTC TTT TAA CAC GGG TCC CTA
His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg Asp>

730          740          750          760
*          *          *          *
TGT GGT TGT AAG CCT TGC ATA TGT ACA GTC CCA GAA GTA TCA TCT GTC
ACA CCA ACA TTC GGA ACG TAT ACA TGT CAG GGT CTT CAT AGT AGA CAG
Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser Val>

770          780          790          800          810
*          *          *          *          *
TTC ATC TTC CCC CCA AAG CCC AAG GAT GTG CTC ACC ATT ACT CTG ACT
AAG TAG AAG CCG GGT TTC GCG TTC CTA CAC GAG TCG TAA TGA GAC TGA
Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu Thr>

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**FIG. 1 C**

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820      830      840      850      860
*      *      *      *      *
CCT AAG GTC ACG TGT GTT GTG GTA GAC ATC AGC AAG GAT GAT CCC GAG
GGA TTC CAG TGC ACA CAA CAC CAT CTG TAG TCG TTC CTA CTA GGG CTC
Pro Lys Val Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro Glu>

      870      880      890      900      910
*      *      *      *      *
GTC CAG TTC AGC TGG TTT GTA GAT GAT GTG GAG GTG CAC ACA GCT CAG
CAG GTC AAG TCG ACC AAA CAT CTA CTA CAC CTC CAC GTG TGT CGA GTC
Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala Gln>

      920      930      940      950      960
*      *      *      *      *
ACG CAA CCC CGG GAG GAG CAG TTC AAC AGC ACT TTC CGC TCA GTC AGT
TGC GTT GGG GCC CTC CTC GTC AAG TTG TCG TGA AAG GCG AGT CAG TCA
Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val Ser>

      970      980      990      1000
*      *      *      *
GAA CTT CCC ATC ATG CAC CAG GAC TGG CTC AAT GGC AAG GAG TTC AAA
CTT GAA GGG TAG TAC GTG GTC CTG ACC GAG TTA CCG TTC CTC AAG TTT
Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn Gly Lys Glu Phe Lys>

1010      1020      1030      1040      1050
*      *      *      *      *
TGC AGG GTC AAC AGT GCA GCT TTC CCT GCC CCC ATC GAG AAA ACC ATC
ACG TCC CAG TTG TCA CCG CGA AAG GGA CCG GCG TAG CTC TTT TGG TAG
Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr Ile>

1060      1070      1080      1090      1100
*      *      *      *      *
TCC AAA ACC AAA GGC AGA CCG AAG GCT CCA CAG GTG TAC ACC ATT CCA
AGG TTT TGG TTT CCG TCT GGC TTC CGA GGT GTC CAC ATG TGG TAA GGT
Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile Pro>

1110      1120      1130      1140      1150
*      *      *      *      *
CCT CCC AAG GAG CAG ATG GCC AAG GAT AAA GTC AGT CTC ACC TGC ATC
GGA GGG TTC CTC GTC TAC CCG TTC CTA TTT CAG TCA GAC TGG ACG TAC
Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val Ser Leu Thr Cys Met>

1160      1170      1180      1190      1200
*      *      *      *      *
ATA ACA GAC TTC TTC CCT GAA GAC ATT ACT GTG GAG TGG CAG TGG AAT
TAT TGT CTG AAG AAG GGA CTT CTG TAA TGA CAC CTC ACC GTC ACC TTA
Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp Asn>

1210      1220      1230      1240
*      *      *      *
GGG CAG CCA CCG GAG AAC TAC AAG AAC ACT CAG CCC ATC ATG GAC ACA
CCC GTC GGT CGC CTC TTG ATG TTC TTG TGA GTC GGG TAG TAC CTC TGT
Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asp Thr>

1250      1260      1270      1280      1290
*      *      *      *      *
GAT GGC TCT TAC TTC GTC TAC AGC AAG CTC AAT GTG CAG AAG AGC AAC
CTA CCC AGA ATG AAG CAG ATG TCG TTC GAG TTA CAC GTC TTC TCG TTC
Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser Asn>

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**FIG. 1 D**

```

1300      1310      1320      1330      1340
  *      *      *      *      *
TGG GAG CCA GGA AAT ACT TTC ACC TGC TCT GTG TTA CAT GAG GGC CTG
ACC CTC CGT CCT TTA TGA AAG TGG ACG AGA CAC AAT GTA CTC CCG GAC
TTP Glu Ala Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly Leu>

1350      1360      1370      1380      1390
  *      *      *      *      *
CAC AAC CAC CAT ACT GAG AAG AGC CTC TCC CAC TCT CCT GGT AAA TG ATC
GTG TTG GTG GTA TGA CTC TTC TCG GAG ACG GTG AGA GGA CCA TTT AC TAG
His Asn His His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys>

1400      1410      1420      1430      1440
  *      *      *      *      *
CCA GTG TCC TTG GAG CCC TCT GGT CCT ACA GGA CTC TGA CAC CTA CCT
GGT CAC AGG AAC CTC GGG AGA CCA GGA TGT CCT GAG ACT GTG GAT GGA

1450      1460      1470      1480
  *      *      *      *
CCA CCC CTC CCT GTA TAA ATA AAG CAC CCA GCA CTG CCT TGG ACC C
GGT GGG GAG GGA CAT ATT TAT TTC GTG GGT CGT GAC GGA ACC TGG G

```

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Sequence of the murine TF8-5G9 light chain cDNA with protein translation. The essential regions of the cDNA are as follows:

**FIG. 2 A**

<u>Nucleotides</u>	<u>Region</u>
1-4	5' untranslated.
5-64	Start codon and leader sequence.
65-385	Variable region.
386-706	Murine kappa constant region.
707-917	3' untranslated region.
918-937	Poly A tail.

Sequence Range: 1 to 937

```

      10      20      30      40
      *      *      *      *
GGA C ATG CGG GCC CCT GCT CAG TTT TTT GGG ATC TTG CTC TGG TTT
CCT G TAC GCC CGG GGA CGA GTC AAA AAA CCC TAG AAC AAC GAG ACC AAA
      Met Arg Ala Pro Ala Gln Phe Phe Gly Ile Leu Leu Leu Trp Phe>

50      60      70      80      90
      *      *      *      *
CCA GGT ATC AGA TGT GAC ATC AAG ATG ACC CAG TCT CCA TCC TCC ATG
GGT CCA TAG TCT ACA CTG TAG TTC TAC TGG GTC AGA GGT AGG AGG TAC
Pro Gly Ile Arg Cys Asp Ile Lys Met Thr Gln Ser Pro Ser Ser Met>

100     110     120     130     140
      *      *      *      *
TAT GCA TCG CTG GGA GAG AGA GTC ACT ATC ACT TGT AAG GCG AGT CAG
ATA CGT AGC GAC CCT CTC TCT CAG TGA TAG TGA ACA TTC CCG TCA GTC
Tyr Ala Ser Leu Gly Glu Arg Val Thr Ile Thr Cys Lys Ala Ser Gln>

150     160     170     180     190
      *      *      *      *
GAC ATT AGA AAG TAT TTA AAC TGG TAC CAG CAG AAA CCA TGG AAA TCT
CTG TAA TCT TTC ATA AAT TTG ACC ATG GTC GTC TTT GGT ACC TTT AGA
Asp Ile Arg Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Trp Lys Ser>

200     210     220     230     240
      *      *      *      *
CCT AAG ACC CTG ATC TAT TAT GCA ACA AGC TTG CCA GAT GGG GTC CCA
GGA TTC TCG GAC TAG ATA ATA CGT TGT TCG AAC CGT CTA CCC CAG GGT
Pro Lys Thr Leu Ile Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val Pro>

250     260     270     280
      *      *      *      *
TCA AGA TTC AGT GGC AGT GGA TCT GCG CAA GAT TAT TCT CTA ACC ATC
AGT TCT AAG TCA CCG TCA CCT AGA CCC GTT CTA ATA AGA GAT TCG TAG
Ser Arg Phe Ser Gly Ser Gly Ser Gly Gln Asp Tyr Ser Leu Thr Ile>

290     300     310     320     330
      *      *      *      *
AGC AGC CTG GAG TCT GAC GAT ACA GCA ACT TAT TAC TGT CTA CAA CAT
TCG TCG GAC CTC AGA CTG CTA TGT CGT TGA ATA ATG ACA GAT GTT GTA
Ser Ser Leu Glu Ser Asp Asp Thr Ala Thr Tyr Tyr Cys Leu Gln His>

```

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**FIG. 2B**

```

340      350      360      370      380
*      *      *      *      *
GGT GAG AGC CCG TAC ACG TTC GGA GGG GGG ACC AAG CTG GAA ATA AAC
CCA CTC TCG GGC ATG TGC AAG CCT CCC CCC TGG TTC GAC CTT TAT TTG
Gly Glu Ser Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Asn>

390      400      410      420      430
*      *      *      *      *
AGG GCT GAT GCT GCA CCA ACT GTA TCC ATC TTC CCA CCA TCC AGT GAG
TCC CGA CTA CGA CGT GGT TGA CAT AGG TAG AAG GGT GGT AGG TCA CTC
Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu>

440      450      460      470      480
*      *      *      *      *
CAG TTA ACA TCT GGA GGT GCC TCA GTC GTG TGC TTC TTG AAC AAC TTC
GTC AAT TGT AGA CCT CCA CGG AGT CAG CAC ACG AAG AAC TTG TTG AAG
Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe>

490      500      510      520
*      *      *      *
TAC CCC AAA GAC ATC AAT GTC AAG TGG AAG ATT GAT GGC AGT GAA CGA
ATG GGG TTT CTG TAG TTA CAG TTC ACC TTC TAA CTA CCG TCA CTT GCT
Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg>

530      540      550      560      570
*      *      *      *      *
CAA AAT GGC GTC CTG AAC AGT TGG ACT GAT CAG GAC AGC AAA GAC AGC
GTT TTA CCG CAG GAC TTG TCA ACC TGA CTA GTC CTG TCG TTT CTG TCG
Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser>

580      590      600      610      620
*      *      *      *      *
ACC TAC AGC ATG AGC AGC ACC CTC ACG TTG ACC AAG GAC GAG TAT GAA
TGG ATG TCG TAC TCG TCG TGG GAG TGC AAC TGG TTC CTG CTC ATA CTT
Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu>

630      640      650      660      670
*      *      *      *      *
CGA CAT AAC AGC TAT ACC TGT GAG GCC ACT CAC AAG ACA TCA ACT TCA
GCT GTA TTG TCG ATA TGG ACA CTC CCG TGA GTG TTC TGT AGT TGA AGT
Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser>

680      690      700      710      720
*      *      *      *      *
CCC ATT GTC AAG AGC TTC AAC AGG AAT GAG TGT TA GAG ACA AAG GTC CTG
GGG TAA CAG TTC TCG AAG TTG TCC TTA CTC ACA AT CTC TGT TTC CAG GAC
Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys>

730      740      750      760      770
*      *      *      *      *
AGA CGC CAC CAC CAG CTC CCC AGC TCC ATC CTA TCT TCC CTT CTA AGG
TCT GCG GTG GTG GTC CAG GGG TCG AGG TAG GAT AGA AGG GAA GAT TCC

780      790      800      810
*      *      *      *
TCT TGG AGG CTT CCC CAC AAG CGA CCT ACC ACT GTT GCG GTG CTC CAA
AGA ACC TCC GAA GGG GTG TTC GCT GGA TGG TGA CAA CGC CAC GAG GTT

```

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**FIG. 2 C**

```

820      830      840      850      860
*          *          *          *          *
ACC TCC TCC CCA CCT CCT TCT CCT CCT CCC TTT CCT TGG CTT TTA
TGG AGG AGG GGT GGA GGA AGA GGA GGA GGG AAA GGA ACC GAA AAT

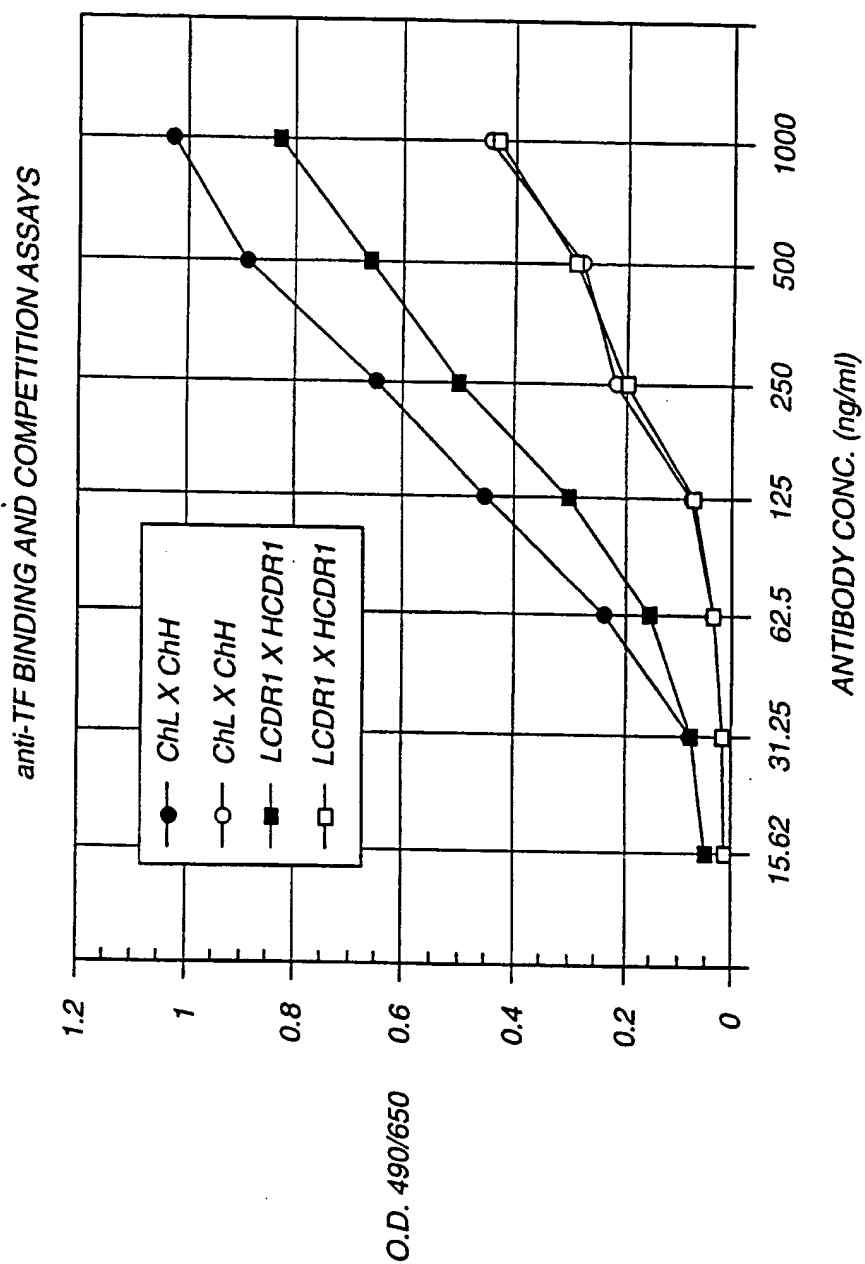
870      880      890      900      910
*          *          *          *          *
TCA TGC TAA TAT TTG CAG AAA ATA TTC AAT AAA GTG AGT CTT TGC ACT
AGT ACG ATT ATA AAC GTC TTT TAT AAG TTA TTT CAC TCA GAA ACG TGA

920      930
*          *
TGA AAA AAA AAA AAA AAA AAA A
ACT TTT TTT TTT TTT TTT TTT T

```

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**FIG. 3**





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**FIG. 4 A**

The pEe6TF8HCDR20 expression vector DNA sequence. The coding regions of the TF8-5G9 CDR-grafted HC gene, TF8HCDR20, are translated.

Sequence Range: 1 to 7073

```

      10      20      30      40
      *      *      *      *
GAA TTC GCC GCC ACC ATG GAA TGG AGC TGG GTC TTT CTC TTC TTC TTG
CTT AAG CCG CCG TGG TAC CTT ACC TCG ACC CAG AAA GAG AAG AAG AAC
      Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu>

50      60      70      80      90
*      *      *      *      *
TCA GTA ACT ACA GGT GTA CAC TCA CAA GTT CAG CTG GTG GAG TCT GGA
AGT CAT TGA TGT CCA CAT GTG AGT GTT CAA GTC GAC CAC CTC AGA CCT
Ser Val Thr Thr Gly Val His Ser Gln Val Gln Leu Val Glu Ser Gly>

100      110      120      130      140
*      *      *      *      *
GGA GGA GTA GTA CAA CCT GGA AGG TCA CTG AGA CTG TCT TGT AAG GCT
CCT CCT CAT CAT GTT GGA CCT TCC AGT GAC TCT GAC AGA ACA TTC CGA
Gly Gly Val Val Gln Pro Gly Arg Ser Leu Arg Leu Ser Cys Lys Ala>

150      160      170      180      190
*      *      *      *      *
AGT GGA TTC AAT ATC AAG GAC TAT TAT ATG CAC TGG GTC AGA CAA GCT
TCA CCT AAG TTA TAG TTC CTG ATA ATA TAC GTG ACC CAG TCT GTT CGA
Ser Gly Phe Asn Ile Lys Asp Tyr Tyr Met His Trp Val Arg Gln Ala>

200      210      220      230      240
*      *      *      *      *
CCT GGA AAA GGA CTC GAG TGG ATA GGT TTA ATT GAT CCT GAG AAT GGT
GGA CCT TTT CCT GAG CTC ACC TAT CCA AAT TAA CTA GGA CTC TTA CCA
Pro Gly Lys Gly Leu Glu Trp Ile Gly Leu Ile Asp Pro Glu Asn Gly>

250      260      270      280
*      *      *      *
AAC ACG ATA TAT GAT CCC AAG TTC CAA GGA AGA TTC ACA ATT TCT GCA
TTG TGC TAT ATA CTA GGG TTC AAG GTT CCT TCT AAG TGT TAA AGA CCT
Asn Thr Ile Tyr Asp Pro Lys Phe Gln Gly Arg Phe Thr Ile Ser Ala>

290      300      310      320      330
*      *      *      *      *
GAC AAC TCT AAG AAT ACA CTG TTC CTG CAG ATG GAC TCA CTC AGA CCT
CTG TTG AGA TTC TTA TGT GAC AAG GAC GTC TAC CTG AGT GAG TCT GGA
Asp Asn Ser Lys Asn Thr Leu Phe Leu Gln Met Asp Ser Leu Arg Pro>

340      350      360      370      380
*      *      *      *      *
GAG GAT ACA GCA GTC TAC TAT TGT GCT AGA GAT AAC AGT TAT TAC TTC
CTC CTA TGT CGT CAG ATC ATA ACA CGA TCT CTA TTG TCA ATA ATG AAG
Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp Asn Ser Tyr Tyr Phe>

390      400      410      420      430
*      *      *      *      *
GAC TAC TGG GGC CAA GGA ACA CCA GTC ACC GTG AGC TCA GCT TCC ACC
CTG ATG ACC CCG GTT CCT TGT GGT CAG TGG CAC TCG AGT CGA AGG TGG
Asp Tyr Trp Gly Gln Gly Thr Pro Val Thr Val Ser Ser Ala Ser Thr>

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**FIG. 4 B**

```

      440      450      460      470      480
      *      *      *      *      *
AAG GGC CCA TCC GTC TTC CCC CTG GCG CCC TGC TCC AGG AGC ACC TCC
TTC CCG GGT AGG CAG AAG GGG GAC CCG GGG ACG AGG TCC TCG TGG AGG
Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser>

      490      500      510      520
      *      *      *      *
GAG AGC ACA GCC GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA
CTC TCG TGT CCG CCG GAC CCG ACG GAC CAG TTC CTG ATG AAG GGG CTT
Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu>

530      540      550      560      570
*      *      *      *      *
CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC
GGC CAC TGC CAC AGC ACC TTG AGT CCG CCG GAC TGG TCG CCG CAC GTG
Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His>

      580      590      600      610      620
      *      *      *      *      *
ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC
TGG AAG GGC CGA CAG GAT GTC AGG AGT CCT GAG ATG AGG GAG TCG TCG
Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser>

      630      640      650      660      670
      *      *      *      *      *
GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACG AAG ACC TAC ACC TGC
CAC CAC TGG CAC GGG AGG TCG TCG AAC CCG TGC TTC TGG ATG TGG ACG
Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys>

      680      690      700      710      720
      *      *      *      *      *
AAC GTA GAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AGA GTT GGT
TTG CAT CTA GTG TTC GGG TCG TTG TGG TTC CAC CTG TTC TCT CAA CCA
Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val>

      730      740      750      760
      *      *      *      *
GAG AGG CCA GCA CAG GCC AGG GAG GGT GTC TGC TGG AAG CCA GGC TCA
CTC TCC GGT CGT GTC CCG TCC CTC CCA CAG ACG ACC TTC GGT CCG AGT

770      780      790      800      810
*      *      *      *      *
GCC CTC CTG CCT GGA CGC ACC CCG GCT GTG CAG CCC CAG CCC AGG GCA
CCG GAG GAC CGA CCT GCG TCG GGC CGA CAC GTC GGG GTC GGG TCC CGT

      820      830      840      850      860
      *      *      *      *      *
GCA AGG CAT GCC CCA TCT GTC TCC TCA CCC GGA GGC CTC TGA CCA CCC
CGT TCC GTA CCG GGT AGA CAG AGG AGT GGG CCT CCG GAG ACT GGT GGG

      870      880      890      900      910
      *      *      *      *      *
CAC TCA TGC TCA GCG AGA GGG TCT TCT GGA TTT TTC CAC CAG GCT CCG
GTG AGT ACG AGT CCC TCT CCC AGA AGA CCT AAA AAG GTG GTC CGA GGC

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**FIG. 4 C**

```

          920          930          940          950          960
          *          *          *          *          *
GGC AGC CAC AGG CTG GAT GCC CCT ACC CCA GGC CCT GCG CAT ACA GGG
CCG TCG GTC TCC GAC CTA CCG GGA TGG GGT CCG GGA CCG GTA TGT CCC

          970          980          990          1000
          *          *          *          *
GCA GGT GCT GCG CTC AGA CCT GCC AAG AGC CAT ATC CCG GAG GAC CCT
CGT CCA CGA CCG GAG TCT GGA CCG TTC TCG GTA TAG GCC CTC CTG GGA

1010          1020          1030          1040          1050
  *          *          *          *          *
GCC CCT GAC CTA AGC CCA CCC CAA AGG CCA AAC TCT CCA CTC CCT CAG
CGG GGA CTG GAT TCG GGT GGG GTT TCC GGT TTG AGA GGT GAG GGA GTC

          1060          1070          1080          1090          1100
          *          *          *          *          *
CTC AGA CAC CTT CTC TCC TCC CAG ATT CGA GTA ACT CCC AAT CTT CTC
GAG TCT GTC GAA GAG AGG AGG GTC TAA GCT CAT TGA GGG TTA GAA GAG

          1110          1120          1130          1140          1150
          *          *          *          *          *
TCT GCA GAG TCC AAA TAT GGT CCC CCA TGC CCA TCA TGC CCA GGT AAG
AGA CGT CTC AGG TTT ATA CCA GGG GGT ACG GGT AGT ACG GGT CCA TTC
      Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro>

          1160          1170          1180          1190          1200
          *          *          *          *          *
CCA ACC CAG GCC TCG CCC TCC AGC TCA AGG CCG GAC AGG TGC CCT AGA
GGT TGG GTC CCG AGC GGG AGG TCG AGT TCC GCC CTG TCC ACG GGA TCT

          1210          1220          1230          1240
          *          *          *          *
GTA GCC TGC ATC CAG GGA CAG GCC CCA GCC GGG TGC TGA CCG ATC CAC
CAT CCG ACG TAG GTC CCT GTC CCG GGT CCG CCC ACG ACT GCG TAG GTG

1250          1260          1270          1280          1290
  *          *          *          *          *
CTC CAT CTC TTC CTC AGC A CCT GAG TTC CTG GCG GGA CCA TCA GTC TTC
GAG GTA GAG AAG GAG TCG T GGA CTC AAG GAC CCC CCT GGT AGT CAG AAG
      Pro Glu Phe Leu Gly Gly Pro Ser Val Phe>

1300          1310          1320          1330          1340
  *          *          *          *          *
CTG TTC CCC CCA AAA CCC AAG GAC ACT CTC ATG ATC TCC CCG ACC CCT
GAC AAG CCG GGT TTT GGG TTC CTG TGA GAG TAC TAG AGG GCC TGG GGA
Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro>

          1350          1360          1370          1380          1390
          *          *          *          *          *
GAG GTC ACG TGC GTC GTC GTC GAC GTC AGC CAG GAA GAC CCC GAG GTC
CTC CAG TCC ACG CAC CAC CAC CTG CAC TCG GTC CTT CTG GCG CTC CAG
Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val>

          1400          1410          1420          1430          1440
          *          *          *          *          *
CAG TTC AAC TGG TAC GTG GAT GCC GTG GAG GTG CAT AAT GCC AAG ACA
GTC AAG TTG ACC ATG CAC CTA CCG CAC CTC CAC GTA TTA CCG TTC TGT
Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr>

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**FIG. 4 D**

```

      1450      1460      1470      1480
      *        *        *        *
AAG CCG CCG GAG GAG CAG TTC AAC AGC ACG TAC CGT GTG GTC AGC GTC
TTC GGC GCC CTC CTC GTC AAG TTG TCG TGC ATG GCA CAC CAG TCG CAG
Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val>

1490      1500      1510      1520      1530
      *        *        *        *
CTC ACC GTC CTG CAC CAG GAC TGG CTG AAC GGC AAG GAG TAC AAG TGC
GAG TGG CAG GAC GTG GTC CTG ACC GAC TTG CCG TTC CTC ATG TTC ACG
Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys>

1540      1550      1560      1570      1580
      *        *        *        *
AAG GTC TCC AAC AAA GGC CTC CCG TCC TCC ATC GAG AAA ACC ATC TCC
TTC CAG AGG TTG TTT CCG GAG GGC AGG AGG TAG CTC TTT TGG TAG AGG
Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser>

      1590      1600      1610      1620      1630
      *        *        *        *
AAA GCC AAA GG TGG GAC CCA CCG GGT GCG AGG GCC ACA TGG ACA GAG GTC
TTT CCG TTT CC ACC CTG GGT GCC CCA CGC TCC CCG TGT ACC TGT CTC CAG
Lys Ala Lys>

      1640      1650      1660      1670      1680
      *        *        *        *
AGC TCG GCC CAC CCT CTG CCC TGG GAG TGA CCG CTG TGC CAA CCT CTG
TCG AGC CCG GTG GGA GAC GGG ACC CTC ACT GGC GAC ACG GTT GGA GAC

      1690      1700      1710      1720      1730
      *        *        *        *
TCC CTA CA GGG CAG CCC CGA GAG CCA CAG GTG TAC ACC CTG CCC CCA TCC
AGG GAT GT CCC GTC GGG GCT CTC GGT GTC CAC ATG TGG GAC GGG GGT AGG
      Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser>

      1740      1750      1760      1770      1780
      *        *        *        *
CAG GAG GAG ATG ACC AAG AAC CAG GTC ACC CTG ACC TGC CTG GTC AAA
GTC CTC CTC TAC TGG TTC TTG GTC CAG TCG GAC TGG ACG GAC CAG TTT
Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys>

      1790      1800      1810      1820
      *        *        *        *
GGC TTC TAC CCC AGC GAC ATC GCC GTG GAG TGG GAG ACC AAT GGG CAG
CCG AAG ATG GGG TCG CTG TAG CCG CAC CTC ACC CTC TCG TTA CCC GTC
Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln>

1830      1840      1850      1860      1870
      *        *        *        *
CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC
GGC CTC TTG TTG ATG TTC TGG TGC GGA GGG CAC GAC CTG AGG CTG CCG
Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly>

1880      1890      1900      1910      1920
      *        *        *        *
TCC TTC TTC CTC TAC AGC AGG CTA ACC GTG GAC AAG AGC AGG TGG CAG
AGG AAG AAG GAG ATG TCG TCC GAT TGG CAC CTG TTC TCG TCC ACC GTC
Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln>

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**FIG. 4 E**

1930 *	1940 *	1950 *	1960 *	1970 *
GAG GGG AAT GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC				
CTC CCC TTA CAG AAG AGT ACG AGG CAC TAC GTA CTC CGA GAC GTG TTG				
Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn>				
1980 *	1990 *	2000 *	2010 *	2020 *
CAC TAC ACA CAG AAG AGC CTC TCC CTG TCT CTG GGT AAA T GAG TGC CAG				
GTG ATG TGT GTC TTC TCG GAG AGG GAC AGA GAC CCA TTT A CTC ACG GTC				
His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys Xxx>				
2030 *	2040 *	2050 *	2060 *	2070 *
GGC CGG CAA GCC CCC GCT CCC CGG GCT CTC GGG GTC GCG CGA GGA TGC				
CCG GCC GTT CGG GGG CGA GGG GCC CGA GAG CCC CAG CGC GCT CCT ACG				
2080 *	2090 *	2100 *	2110 *	
TTG GCA CGT ACC CCG TCT ACA TAC TTC CCA GGC ACC CAG CAT GGA AAT				
AAC CGT GCA TGG GGC AGA TGT ATG AAG GGT CCG TGG GTC GTA CCT TTA				
2120 *	2130 *	2140 *	2150 *	2160 *
AAA GCA CCC ACC ACT GCC CTG GGC CCC TGT GAG ACT GTG ATG GTT CTT				
TTT CGT GGG TGG TGA CGG GAC CCG GGG ACA CTC TGA CAC TAC CAA GAA				
2170 *	2180 *	2190 *	2200 *	2210 *
TCC ACG GGT CAG GCC GAG TCT GAG GCC TGA GTG ACA TGA GGG AGG CAG				
AGG TGC CCA GTC CGG CTC AGA CTC CGG ACT CAC TGT ACT CCC TCC GTC				
2220 *	2230 *	2240 *	2250 *	2260 *
AGC GGG TCC CAC TGT CCC CAC ACT GGC CCA GGC TGT GCA GGT GTG CCT				
TCG CCC AGG GTG ACA GGG GTG TGA CCG GGT CCG ACA CGT CCA CAC GGA				
2270 *	2280 *	2290 *	2300 *	2310 *
GGG CCA CCT AGG GTG GGG CTC AGC CAG GGG CTG CCC TCG GCA GGG TGG				
CCC GGT GGA TCC CAC CCC GAG TCG GTC CCC GAC GGG AGC CGT CCC ACC				
2320 *	2330 *	2340 *	2350 *	
GGG ATT TGC CAG CGT GGC CCT CCC TCC AGC AGC AGG ACT CTA GAG GAT				
CCC TAA ACC GTC GCA CCG GGA GGG AGG TCG TCG TCC TGA GAT CTC CTA				
2360 *	2370 *	2380 *	2390 *	2400 *
CAT AAT CAG CCA TAC CAC ATT TGT AGA GGT TTT ACT TGC TTT AAA AAA				
GTA TTA GTC GGT ATG GTG TAA ACA TCT CCA AAA TGA ACG AAA TTT TTT				
2410 *	2420 *	2430 *	2440 *	2450 *
CCT CCC ACA CCT CCC CCT GAA CCT GAA ACA TAA AAT GAA TGC AAT TGT				
CGA GGG TGT GGA GGG GGA CTT GGA CTT TGT ATT TTA CTT ACG TTA ACA				

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**FIG. 4 F**

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      2460      2470      2480      2490      2500
      *        *        *        *        *
TGT TGT TAA CTT GTT TAT TGC AGC TTA TAA TGG TTA CAA ATA AAG CAA
ACA ACA ATT GAA CAA ATA ACG TCG AAT ATT ACC AAT GTT TAT TTC GTT

      2510      2520      2530      2540      2550
      *        *        *        *        *
TAG CAT CAC AAA TTT CAC AAA TAA AGC ATT TTT TTC ACT GCA TTC TAG
ATC GTA GTG TTT AAA GTG TTT ATT TCG TAA AAA AAG TGA CGT AAG ATC

      2560      2570      2580      2590
      *        *        *        *
TTG TGG TTT GTC CAA ACT CAT CAA TGT ATC TTA TCA TGT CTG GAT CCT
AAC ACC AAA CAG GTT TGA GTA GTT ACA TAG AAT AGT ACA GAC CTA GGA

2600      2610      2620      2630      2640
*        *        *        *        *
CTA CGC CGG ACG CAT CGT GGC CGG CAT CAC CGG CGC CAC AGG TGC GGT
GAT GCG GCC TGC GTA GCA CCG GCC GTA GTG GCC GCG GTG TCC ACC CCA

      2650      2660      2670      2680      2690
      *        *        *        *        *
TGC TGG CGC CTA TAT CGC CGA CAT CAC CGA TGG GGA AGA TCG GGC TCG
ACG ACC GCG GAT ATA GCG GCT GTA GTG GCT ACC CCT TCT AGC CCG AGC

      2700      2710      2720      2730      2740
      *        *        *        *        *
CCA CTT CGG GCT CAT GAG CGC TTG TTT CGG CGT GGG TAT GGT GGC AGG
GGT GAA GCC CGA GTA CTC GCG AAC AAA GCC GCA CCC ATA CCA CCG TCC

      2750      2760      2770      2780      2790
      *        *        *        *        *
CCC GTG GCC GGG GGA CTG TTG GGC GCC ATC TCC TTG CAT GCA CCA TTC
GGG CAC CCG CCC CCT GAC AAC CCG CCG TAG AGG AAC GTA CGT GGT AAG

      2800      2810      2820      2830
      *        *        *        *
CTT GCG GCG GCG GTG CTC AAC GGC CTC AAC CTA CTA CTG GGC TGC TTC
GAA CCG CCG CCG CAC GAG TTG CCG GAG TTG GAT GAT GAC CCG ACG AAG

2840      2850      2860      2870      2880
*        *        *        *        *
CTA ATG CAG GAG TCG CAT AAG GGA GAG CGT CGA CCT CGG GCC GCG TTG
GAT TAC GTC CTC AGC GTA TTC CCT CTC GCA GCT GGA GCC CCG CGC AAC

      2890      2900      2910      2920      2930
      *        *        *        *        *
CTG GCG TTT TTC CAT AGG CTC CGC CCC CCT GAC GAG CAT CAC AAA AAT
GAC CCG AAA AAG GTA TCC GAG GCG GGG GGA CTG CTC GTA GTG TTT TTA

      2940      2950      2960      2970      2980
      *        *        *        *        *
CGA CGC TCA AGT CAG AGG TGG CGA AAC CCG ACA GGA CTA TAA AGA TAC
GCT CCG AGT TCA GTC TCC ACC GCT TTG GGC TGT CCT GAT ATT TCT ATG

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**FIG. 4 G**

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      2990      3000      3010      3020      3030
      *      *      *      *      *
CAG GCG TTT CCC CCT GGA AGC TCC CTC GTG CGC TCT CCT GTT CCG ACC
GTC CGC AAA GGG GGA CCT TCG AGG GAG CAC GCG AGA GGA CAA GGC TGG

      3040      3050      3060      3070
      *      *      *      *
CTG CCG CTT ACC GGA TAC CTG TCC GCC TTT CTC CCT TCG GGA AGC GTG
GAC GGC GAA TGG CCT ATG GAC AGG CGG AAA GAG GGA AGC CCT TCG CAC

3080      3090      3100      3110      3120
*      *      *      *      *
GCG CTT TCT CAA TGC TCA CGC TGT AGG TAT CTC AGT TCG GTG TAG GTC
CGC GAA AGA GTT ACG AGT CGC ACA TCC ATA GAG TCA AGC CAC ATC CAG

      3130      3140      3150      3160      3170
      *      *      *      *      *
GTT CGC TCC AAG CTG GGC TGT GTG CAC GAA CCC CCC GTT CAG CCC GAC
CAA GCG AGG TTC GAC CCG ACA CAC GTG CTT GGG GGG CAA GTC GGG CTG

      3180      3190      3200      3210      3220
      *      *      *      *      *
CGC TGC GCC TTA TCC GGT AAC TAT CGT CTT GAG TCC AAC CCG GTA AGA
GCG ACG CGG AAT AGG CCA TTG ATA GCA GAA CTC AGG TTG GGC CAT TCT

      3230      3240      3250      3260      3270
      *      *      *      *      *
CAC GAC TTA TCG CCA CTG GCA GCA GCC ACT GGT AAC AGG ATT AGC AGA
GTG CTG AAT AGC GGT GAC CGT CGT CGG TGA CCA TTG TCC TAA TCG TCT

      3280      3290      3300      3310
      *      *      *      *
GCG AGG TAT GTA GGC GGT GCT ACA GAG TTC TTG AAG TGG TGG CCT AAC
CGC TCC ATA CAT CCG CCA CGA TGT CTC AAG AAC TTC ACC ACC GGA TTG

3320      3330      3340      3350      3360
*      *      *      *      *
TAC GGC TAC ACT AGA AGG ACA GTA TTT GGT ATC TGC GCT CTG CTG AAG
ATG CCG ATG TGA TCT TCC TGT CAT AAA CCA TAG ACG CGA GAC GAC TTC

      3370      3380      3390      3400      3410
      *      *      *      *      *
CCA GTT ACC TTC GGA AAA AGA GTT GGT AGC TCT TGA TCC GGC AAA CAA
GGT CAA TGG AAG CCT TTT TCT CAA CCA TCG AGA ACT AGG CCG TTT GTT

      3420      3430      3440      3450      3460
      *      *      *      *      *
ACC ACC GCT GGT AGC GGT TTT TTT GTT TGC AAG CAG CAG ATT ACG
TGG TGG CGA CCA TCG CCA CCA AAA AAA CAA ACG TTC GTC GTC TAA TGC

      3470      3480      3490      3500      3510
      *      *      *      *      *
CGC AGA AAA AAA GGA TCT CAA GAA GAT CCT TTG ATC TTT TCT ACG GGC
GCG TCT TTT TTT CCT AGA GTT CTT CTA GGA AAC TAG AAA AGA TGC CCC

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**FIG. 4 H**

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      3520      3530      3540      3550
      *      *      *      *
TCT GAC GCT CAG TGG AAC GAA AAC TCA CGT TAA GGG ATT TTG GTC ATG
AGA CTC CGA GTC ACC TTG CTT TTG AGT GCA ATT CCC TAA AAC CAG TAC

3560      3570      3580      3590      3600
      *      *      *      *      *
AGA TTA TCA AAA AGG ATC TTC ACC TAG ATC CTT TTA AAT TAA AAA TGA
TCT AAT AGT TTT TCC TAG AAG TGG ATC TAG GAA AAT TTA ATT TTT ACT

      3610      3620      3630      3640      3650
      *      *      *      *      *
AGT TTT AAA TCA ATC TAA AGT ATA TAT GAG TAA ACT TGG TCT GAC AGT
TCA AAA TTT AGT TAG ATT TCA TAT ATA CTC ATT TGA ACC AGA CTG TCA

      3660      3670      3680      3690      3700
      *      *      *      *      *
TAC CAA TGC TTA ATC AGT GAG GCA CCT ATC TCA GCG ATC TGT CTA TTT
ATG GTT ACG AAT TAG TCA CTC CGT GGA TAG AGT CGC TAG ACA GAT AAA

      3710      3720      3730      3740      3750
      *      *      *      *      *
CGT TCA TCC ATA GTT GCC TGA CTC CCC GTC GTG TAG ATA ACT ACG ATA
GCA AGT AGG TAT CAA CGG ACT GAG GGG CAG CAC ATC TAT TGA TGC TAT

      3760      3770      3780      3790
      *      *      *      *
CGG GAG GGC TTA CCA TCT GGC CCC AGT GCT GCA ATG ATA CCG CGA GAC
GCC CTC CCG AAT GGT AGA CCG GGG TCA CGA CGT TAC TAT GGC GCT CTG

3800      3810      3820      3830      3840
      *      *      *      *      *
CCA CGC TCA CCG GCT CCA GAT TTA TCA GCA ATA AAC CAG CCA GCC GGA
GGT GCG AGT GGC CGA GGT CTA AAT AGT CGT TAT TTG GTC GGT CCG CCT

      3850      3860      3870      3880      3890
      *      *      *      *      *
AGG GCC GAG CCG AGA AGT GGT CCT GCA ACT TTA TCC GCC TCC ATC CAG
TCC CCG CTC CCG TCT TCA CCA GGA CGT TGA AAT AGG CCG AGG TAG GTC

      3900      3910      3920      3930      3940
      *      *      *      *      *
TCT ATT AAT TGT TGC CCG GAA GCT AGA GTA AGT AGT TCG CCA GTT AAT
AGA TAA TTA ACA ACG GCC CTT CGA TCT CAT TCA TCA AGC GGT CAA TTA

      3950      3960      3970      3980      3990
      *      *      *      *      *
AGT TTG CCG AAC GTT GTT GCC ATT GCT ACA GGC ATC GTG GTG TCA CCG
TCA AAC GCG TTG CAA CAA CCG TAA CGA TGT CCG TAG CAC CAC AGT GCG

      4000      4010      4020      4030
      *      *      *      *
TCG TCG TTT GGT ATG GCT TCA TTC AGC TCC GGT TCC CAA CGA TCA AGG
AGC AGC AAA CCA TAC CGA AGT AAG TCG AGG CCA AGG GTT GCT AGT TCC

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**FIG. 4 I**

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4040      4050      4060      4070      4080
      *      *      *      *      *
CGA GTT ACA TGA TCC CCC ATG TTG TGC AAA AAA GCG GTT AGC TCC TTC
GCT CAA TGT ACT AGG GGG TAC AAC ACG TTT TTT CGC CAA TCG AGG AAG

4090      4100      4110      4120      4130
      *      *      *      *      *
GGT CCT CCG ATC GTT GTC AGA AGT AAG TTG GCC GCA GTG TTA TCA CTC
CCA GGA GGC TAG CAA CAG TCT TCA TTC AAC CGG CGT CAC AAT AGT GAG

4140      4150      4160      4170      4180
      *      *      *      *      *
ATG GTT ATG GCA GCA CTG CAT AAT TCT CTT ACT GTC ATG CCA TCC GTA
TAC CAA TAC CGT CGT GAC GTA TTA AGA GAA TGA CAG TAC GGT AGG CAT

4190      4200      4210      4220      4230
      *      *      *      *      *
AGA TGC TTT TCT GTG ACT GGT GAG TAC TCA ACC AAG TCA TTC TGA GAA
TCT ACG AAA AGA CAC TGA CCA CTC ATG AGT TGG TTC AGT AAG ACT CTT

4240      4250      4260      4270
      *      *      *      *
TAG TGT ATG CCG CGA CCG AGT TGC TCT TGC CCG GCG TCA ACA CCG GAT
ATC ACA TAC GCC GCT GGC TCA ACG AGA ACG GGC CGC AGT TGT GCC CTA

4280      4290      4300      4310      4320
      *      *      *      *      *
AAT ACC GCG CCA CAT AGC AGA ACT TTA AAA GTG CTC ATC ATT GGA AAA
TTA TGG CGC GGT GTA TCG TCT TGA AAT TTT CAC GAG TAG TAA CCT TTT

4330      4340      4350      4360      4370
      *      *      *      *      *
CGT TCT TCG GGG CGA AAA CTC TCA AGG ATC TTA CCG CTG TTG AGA TCC
GCA AGA AGC CCC GCT TTT GAG AGT TCC TAG AAT GGC GAC AAC TCT AGG

4380      4390      4400      4410      4420
      *      *      *      *      *
AGT TCG ATG TAA CCC ACT CGT GCA CCC AAC TGA TCT TCA GCA TCT TTT
TCA AGC TAC ATT GGG TGA GCA CGT GGG TTG ACT AGA AGT CGT AGA AAA

4430      4440      4450      4460      4470
      *      *      *      *      *
ACT TTC ACC AGC GTT TCT GGG TGA GCA AAA ACA GGA AGG CAA AAT GCC
TGA AAG TGG TCG CAA AGA CCC ACT CGT TTT TGT CCT TCC GTT TTA CGG

4480      4490      4500      4510
      *      *      *      *
GCA AAA AAG GGA ATA AGG GCG ACA CGG AAA TGT TGA ATA CTC ATA CTC
CGT TTT TTC CCT TAT TCC CGC TGT GCC TTT ACA ACT TAT GAG TAT GAG

4520      4530      4540      4550      4560
      *      *      *      *      *
TTC CTT TTT CAA TAT TAT TGA AGC ATT TAT CAG GGT TAT TGT CTC ATG
AAG GAA AAA GTT ATA ATA ACT TCG TAA ATA GTC CCA ATA ACA GAG TAC

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**FIG. 4 J**

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4570      4580      4590      4600      4610
*         *         *         *         *
AGC GGA TAC ATA TTT GAA TGT ATT TAG AAA AAT AAA CAA ATA GGC GTT
TCG CCT ATG TAT AAA CTT ACA TAA ATC TTT TTA TTT GTT TAT CCC CAA

4620      4630      4640      4650      4660
*         *         *         *         *
CCG CGC ACA TTT CCC CGA AAA GTG CCA CCT GAC GTC TAA GAA ACC ATT
GGC GCG TGT AAA GGG GCT TTT CAC GGT GGA CTG CAG ATT CTT TCG TAA

4670      4680      4690      4700      4710
*         *         *         *         *
ATT ATC ATG ACA TTA ACC TAT AAA AAT AGG CGT ATC ACC AGG CCC TGA
TAA TAG TAC TGT AAT TGG ATA TTT TTA TCC GCA TAG TGC TCC GGG ACT

4720      4730      4740      4750
*         *         *         *
TGG CTC TTT GCG GCA CCC ATC GTT CGT AAT GTT CCG TCG CAC CGA GGA
ACC GAG AAA CGC CGT GCG TAG CAA GCA TTA CAA GGC ACC GTG GCT CCT

4760      4770      4780      4790      4800
*         *         *         *         *
CAA CCC TCA AGA GAA AAT GTA ATC ACA CTG GCT CAC CTT CCG GTG GGC
GTT GGG AGT TCT CTT TTA CAT TAG TGT GAC CGA GTG GAA GCC CAC CCG

4810      4820      4830      4840      4850
*         *         *         *         *
CTT TCT GCG TTT ATA AGG AGA CAC TTT ATG TTT AAG AAG GTT GGT AAA
GAA AGA CGC AAA TAT TCC TCT GTG AAA TAC AAA TTC TTC CAA CCA TTT

4860      4870      4880      4890      4900
*         *         *         *         *
TTC CTT GCG GCT TTG GCA GCC AAG CTA GAG ATC TCT AGC TTC GTG TCA
AAG GAA CGC CGA AAC CGT CGG TTC GAT CTC TAG AGA TCG AAG CAC AGT

4910      4920      4930      4940      4950
*         *         *         *         *
AGG ACC GTG ACT GCA GTG AAT AAT AAA ATG TGT GTT TGT CCG AAA TAC
TCC TGC CAC TGA CGT CAC TTA TTA TTT TAC ACA CAA ACA GGC TTT ATG

4960      4970      4980      4990
*         *         *         *
GCG TTT TGA GAT TTC TGT GCG CGA CTA AAT TCA TGT CCG GCG ATA GTG
CGC AAA ACT CTA AAG ACA GCG GCT GAT TTA AGT ACA GCG CGC TAT CAC

5000      5010      5020      5030      5040
*         *         *         *         *
GTG TTT ATC GCC GAT AGA GAT GGC GAT ATT GGA AAA ATC GAT ATT TGA
CAC AAA TAG CGC CTA TCT CTA CCG CTA TAA CCT TTT TAG CTA TAA ACT

5050      5060      5070      5080      5090
*         *         *         *         *
AAA TAT GGC ATA TTG AAA ATG TCG CCG ATG TGA GTT TCT GTG TAA CTG
TTT ATA CCG TAT AAC TTT TAC AGC GGC TAC ACT CAA AGA CAC ATT GAC

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**FIG. 4 K**

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      5100      5110      5120      5130      5140
      *        *        *        *        *
ATA TCG CCA TTT TTC CAA AAG TGA TTT TTG GGC ATA CGC GAT ATC TGG
TAT AGC GGT AAA AAG GTT TTC ACT AAA AAC CCG TAT GCG CTA TAG ACC

      5150      5160      5170      5180      5190
      *        *        *        *        *
CGA TAG CGC TTA TAT CGT TTA CGG GGG ATG GCG ATA GAC GAC TTT GGT
GCT ATC GCG AAT ATA GCA AAT GCC CCC TAC CCG TAT CTG CTG AAA CCA

      5200      5210      5220      5230
      *        *        *        *
GAC TTG GGC GAT TCT GTG TGT CGC AAA TAT CGC AGT TTC GAT ATA GGT
CTG AAC CCG CTA AGA CAC ACA GCG TTT ATA GCG TCA AAG CTA TAT CCA

5240      5250      5260      5270      5280
      *        *        *        *        *
GAC AGA CGA TAT GAG GCT ATA TCG CCG ATA GAG GCG ACA TCA AGC TGG
CTG TCT GCT ATA CTC CGA TAT AGC GGC TAT CTC CCG TGT AGT TCG ACC

      5290      5300      5310      5320      5330
      *        *        *        *        *
CAC ATG GCC AAT GCA TAT CGA TCT ATA CAT TGA ATC AAT ATT GGC CAT
GTG TAC CCG TTA CGT ATA GCT AGA TAT GTA ACT TAG TTA TAA CCG GTA

      5340      5350      5360      5370      5380
      *        *        *        *        *
TAG CCA TAT TAT TCA TTG GTT ATA TAG CAT AAA TCA ATA TTG GCT ATT
ATC GGT ATA ATA AGT AAC CAA TAT ATC GTA TTT AGT TAT AAC CGA TAA

      5390      5400      5410      5420      5430
      *        *        *        *        *
GGC CAT TGC ATA CGT TGT ATC CAT ATC ATA ATA TGT ACA TTT ATA TTG
CCG GTA ACG TAT GCA ACA TAG GTA TAG TAT TAT ACA TGT AAA TAT AAC

      5440      5450      5460      5470
      *        *        *        *
GCT CAT GTC CAA CAT TAC CGC CAT GTT GAC ATT GAT TAT TGA CTA GTT
CGA GTA CAG GTT GTA ATG GCG GTA CAA CTG TAA CTA ATA ACT GAT CAA

5480      5490      5500      5510      5520
      *        *        *        *        *
ATT AAT AGT AAT CAA TTA CGG GGT CAT TAG TTC ATA GCC CAT ATA TGG
TAA TTA TCA TTA GTT AAT GCC CCA GTA ATC AAG TAT CCG GTA TAT ACC

      5530      5540      5550      5560      5570
      *        *        *        *        *
AGT TCC GCG TTA CAT AAC TTA CGG TAA ATG GCC CCG CTG GCT GAC CCG
TCA AGG CCG AAT GTA TTG AAT GCC ATT TAC CCG GCG GAC CGA CTG GCG

      5580      5590      5600      5610      5620
      *        *        *        *        *
CCA ACG ACC CCC GCC CAT TGA CGT CAA TAA TGA CGT ATG TTC CCA TAG
GGT TGC TGG GGG CCG GTA ACT GCA GTT ATT ACT GCA TAC AAG GGT ATC

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**FIG. 4 L**

```

      5630      5640      5650      5660      5670
      *      *      *      *      *
TAA CGC CAA TAG GGA CTT TCC ATT GAC GTC AAT GGG TGG AGT ATT TAC
ATT GCG GTT ATC CCT GAA AGG TAA CTG CAG TTA CCC ACC TCA TAA ATG

      5680      5690      5700      5710
      *      *      *      *
GGT AAA CTG CCC ACT TGG CAG TAC ATC AAG TGT ATC ATA TGC CAA GTA
CCA TTT GAC GGG TGA ACC GTC ATG TAG TTC ACA TAG TAT ACG GTT CAT

5720      5730      5740      5750      5760
*      *      *      *      *
CGC CCC CTA TTG ACG TCA ATG ACG GTA AAT GGC CCG CCT GGC ATT ATG
GCG GGG GAT AAC TGC AGT TAC TGC CAT TTA CCG GGC GGA CCG TAA TAC

      5770      5780      5790      5800      5810
      *      *      *      *      *
CCC AGT ACA TGA CCT TAT GGG ACT TTC CTA CTT GGC AGT ACA TCT ACG
GGG TCA TGT ACT GGA ATA CCC TGA AAG GAT GAA CCG TCA TGT AGA TGC

      5820      5830      5840      5850      5860
      *      *      *      *      *
TAT TAG TCA TCG CTA TTA CCA TGG TGA TGC GGT TTT GGC AGT ACA TCA
ATA ATC AGT AGC GAT AAT GGT ACC ACT ACG CCA AAA CCG TCA TGT AGT

      5870      5880      5890      5900      5910
      *      *      *      *      *
ATG GGC GTG GAT AGC GGT TTG ACT CAC GGG GAT TTC CAA GTC TCC ACC
TAC CCG CAC CTA TCG CCA AAC TGA GTG CCC CTA AAG GTT CAG AGG TGG

      5920      5930      5940      5950
      *      *      *      *
CCA TTG ACG TCA ATG GGA GTT TGT TTT GGC ACC AAA ATC AAC GGC ACT
GGT AAC TGC AGT TAC CCT CAA ACA AAA CCG TGG TTT TAG TTG CCC TGA

5960      5970      5980      5990      6000
*      *      *      *      *
TTC CAA AAT GTC GTA ACA ACT CCG CCC CAT TGA CCG AAA TGG CCG GTA
AAG GTT TTA CAG CAT TGT TGA GGC GGG GTA ACT GCG TTT ACC CCG CAT

      6010      6020      6030      6040      6050
      *      *      *      *      *
GGC GTG TAC GGT GGG AGG TCT ATA TAA GCA GAG CTC GTT TAG TGA ACC
CCG CAC ATG CCA CCC TCC AGA TAT ATT CGT CTC GAG CAA ATC ACT TGG

      6060      6070      6080      6090      6100
      *      *      *      *      *
GTC AGA TCG CCT GGA GAC GCC ATC CAC GCT GTT TTG ACC TCC ATA GAA
CAG TCT AGC GGA CCT CTG CCG TAG GTG CGA CAA AAC TGG AGG TAT CTT

      6110      6120      6130      6140      6150
      *      *      *      *      *
GAC ACC GGG ACC GAT CCA GCC TCC GCG GCC GGG AAC GGT GCA TTG GAA
CTG TGG CCC TGG CTA GGT CCG AGG CCG CCG CCC TTG CCA CGT AAC CTT

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**FIG. 4 M**

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      6160      6170      6180      6190
      *        *        *        *
CGC GGA TTC CCC GTG CCA AGA GTG ACG TAA GTA CCG CCT ATA GAG TCT
GCG CCT AAG GGG CAC GGT TCT CAC TGC ATT CAT GGC GGA TAT CTC AGA

6200      6210      6220      6230      6240
      *        *        *        *
ATA GGC CCA CCC CCT TGG CTT CTT ATG CAT GCT ATA CTG TTT TTG GCT
TAT CCG GGT GGG CGA ACC GAA GAA TAC GTA CGA TAT GAC AAA AAC CGA

      6250      6260      6270      6280      6290
      *        *        *        *
TGG GGT CTA TAC ACC CCC GCT TCC TCA TGT TAT AGG TGA TGG TAT AGC
ACC CCA GAT ATG TGG GGG CGA AGG AGT ACA ATA TCC ACT ACC ATA TCG

      6300      6310      6320      6330      6340
      *        *        *        *
TTA GCC TAT AGG TGT GGG TTA TTG ACC ATT ATT GAC CAC TCC CCT ATT
AAT CCG ATA TCC ACA CCC AAT AAC TGG TAA TAA CTG GTG AGG GGA TAA

      6350      6360      6370      6380      6390
      *        *        *        *
GGT GAC GAT ACT TTC CAT TAC TAA TCC ATA ACA TGG CTC TTT GCC ACA
CCA CTG CTA TGA AAG GTA ATG ATT AGG TAT TGT ACC GAG AAA CGG TGT

      6400      6410      6420      6430
      *        *        *        *
ACT CTC TTT ATT GGC TAT ATG CCA ATA CAC TGT CCT TCA GAG ACT GAC
TGA GAG AAA TAA CCG ATA TAC GGT TAT GTG ACA GGA AGT CTC TGA CTG

6440      6450      6460      6470      6480
      *        *        *        *
ACG GAC TCT GTA TTT TTA CAG GAT GGG GTC TCA TTT ATT ATT TAC AAA
TGC CTG AGA CAT AAA AAT GTC CTA CCC CAG AGT AAA TAA TAA ATG TTT

      6490      6500      6510      6520      6530
      *        *        *        *
TTC ACA TAT ACA ACA CCA CCG TCC CCA GTG CCC GCA GTT TTT ATT AAA
AAG TGT ATA TGT TGT GGT GGC AGG GGT CAC GGG CGT CAA AAA TAA TTT

      6540      6550      6560      6570      6580
      *        *        *        *
CAT AAC GTG GGA TCT CCA CGC GAA TCT CCG GTA CGT GTT CCG GAC ATG
GTA TTG CAC CCT AGA GGT GCG CTT AGA GCC CAT GCA CAA GGC CTG TAC

      6590      6600      6610      6620      6630
      *        *        *        *
GGC TCT TCT CCG GTA GCG GCG GAG CTT CTA CAT CCG AGC CCT GCT CCC
CCG AGA AGA GGC CAT CGC CGC CTC GAA GAT GTA GGC TCG GGA CGA GGG

      6640      6650      6660      6670
      *        *        *        *
ATG CCT CCA GCG ACT CAT GGT CCG TCG GCA GCT CCT TGC TCC TAA CAG
TAC GGA GGT CCG TGA GTA CCA GCG AGC CGT CGA GGA ACG AGG ATT GTC

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**FIG. 4 N**

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6680      6690      6700      6710      6720
  *      *      *      *      *
TGG AGG CCA GAC TTA GGC ACA GCA CGA TGC CCA CCA CCA CCA GTG TGC
ACC TCC GGT CTG AAT CCG TGT CGT GCT ACG GGT GGT GGT GGT CAC ACG

6730      6740      6750      6760      6770
  *      *      *      *      *
CGC ACA AGG CCG TGG CCG TAG GGT ATG TGT CTG AAA ATG AGC TCG GGG
GCG TGT TCC GGC ACC GCC ATC CCA TAC ACA GAC TTT TAC TCG AGC CCC

6780      6790      6800      6810      6820
  *      *      *      *      *
AGC GGG CTT GCA CCG CTG ACG CAT TTG GAA GAC TTA AGG CAG CCG CAG
TCG CCC GAA CGT GGC GAC TGC GTA AAC CTT CTG AAT TCC GTC GCC GTC

6830      6840      6850      6860      6870
  *      *      *      *      *
AAG AAG ATG CAG GCA GCT GAG TTG TTG TGT TCT GAT AAG AGT CAG AGG
TTC TTC TAC GTC CGT CGA CTC AAC AAC ACA AGA CTA TTC TCA GTC TCC

6880      6890      6900      6910
  *      *      *      *
TAA CTC CCG TTG CCG TCC TGT TAA CCG TGG AGG GCA GTG TAG TCT GAG
ATT CAG GGC AAC GCC ACG ACA ATT GCC ACC TCC CGT CAC ATC AGA CTC

6920      6930      6940      6950      6960
  *      *      *      *      *
CAG TAC TCG TTG CTG CCG CCG GCG CCA CCA GAC ATA ATA GCT GAC AGA
GTC ATG AGC AAC GAC GGC GCG CCG GGT GGT CTG TAT TAT CGA CTG TCT

6970      6980      6990      7000      7010
  *      *      *      *      *
CTA ACA GAC TGT TCC TTT CCA TGG GTC TTT TCT GCA GTC ACC GTC CTT
GAT TGT CTG ACA AGG AAA GGT ACC CAG AAA AGA CGT CAG TGG CAG GAA

7020      7030      7040      7050      7060
  *      *      *      *      *
GAC ACG AAG CTT GGG CTG CAG GTC GAT CGA CTC TAG AGG ATC GAT CCC
CTG TGC TTC GAA CCC GAC GTC CAG CTA GCT GAG ATC TCC TAG CTA GGG

7070
  *
CGG GCG AGC TC
GCC CCG TCG AG

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**FIG. 5 A**

The pE $\epsilon$ 12TF8LCDR3 expression vector DNA sequence. The coding regions of the TF8-5G9 CDR-grafted LC gene, TF8LCDR3, are translated.

Sequence Range: 1 to 7864

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      10      20      30      40      50
      *      *      *      *      *
AAT TCA CC ATG GGT GTG CCA ACT CAG GTA TTA GGA TTA CTG CTG CTG TCG
TTA AGT GG TAC CCA CAC GGT TGA GTC CAT AAT CCT AAT GAC GAC GAC ACC
      Met Gly Val Pro Thr Gln Val Leu Gly Leu Leu Leu Leu Trp>

      60      70      80      90
      *      *      *      *
CTT ACA GAT GCA AGA TGT GAT ATC CAA ATG ACA CAA TCT CCT TCT TCT
GAA TGT CTA CGT TCT ACA CTA TAG GTT TAC TGT GTT AGA GGA AGA AGA
Leu Thr Asp Ala Arg Cys Asp Ile Gln Met Thr Gln Ser Pro Ser Ser>

100      110      120      130      140
      *      *      *      *      *
CTA AGT GCT TCT GTC GGA GAT AGA GTA ACA ATT ACA TGT AAG GCG AGT
GAT TCA CGA AGA CAG CCT CTA TCT CAT TGT TAA TGT ACA TTC CGC TCA
Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser>

      150      160      170      180      190
      *      *      *      *      *
CAG GAC ATT AGA AAG TAT TTA AAC TGG TAT CAG CAA AAA CCT GGG AAG
GTC CTG TAA TCT TTC ATA AAT TTG ACC ATA GTC GTT TTT GGA CCC TTC
Gln Asp Ile Arg Lys Tyr Leu Asn Trp Tyr Gln Gln Lys Pro Gly Lys>

      200      210      220      230      240
      *      *      *      *      *
GCT CCT AAG CTA CTG ATT TAT TAT GCA ACA AGT TTG GCA GAT GGA GTA
CGA GGA TTC GAT GAC TAA ATA ATA CGT TGT TCA AAC CGT CTA CCT CAT
Ala Pro Lys Leu Leu Ile Tyr Tyr Ala Thr Ser Leu Ala Asp Gly Val>

      250      260      270      280      290
      *      *      *      *      *
CCT TCT AGA TTT TCT GGT TCT GGC TCT GGA ACA GAC TAC ACA TTC ACA
CGA AGA TCT AAA AGA CCA AGA CCG AGA CCT TGT CTG ATG TGT AAG TGT
Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Tyr Thr Phe Thr>

      300      310      320      330
      *      *      *      *
ATT TCT TCT CTC CAA CCT GAG GAC ATT GCT ACA TAC TAC TGC CTA CAA
TAA AGA AGA GAG GTT GGA CTC CTG TAA CGA TGT ATG ATG ACG GAT GTT
Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Leu Gln>

340      350      360      370      380
      *      *      *      *      *
CAT GGT GAG AGT CCG TAT ACA TTT GGA CAA GGA ACA AAA CTA GAG ATC
GTA CCA CTC TCA GGC ATA TGT AAA CCT GTT CCT TGT TTT GAT CTC TAG
His Gly Glu Ser Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile>

      390      400      410      420      430
      *      *      *      *      *
ACA AGA ACT GTT GCG GCG CCG TCT GTC TTC ATC TTC CCG CCA TCT GAT
TGT TCT TGA CAA CCG CCG GGC AGA CAG AAG TAG AAG GGC GGT AGA CTA
Thr Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp>

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**FIG. 5 B**

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      440      450      460      470      480
      *      *      *      *      *
GAG CAG TTG AAA TCT GGA ACT GCC TCT GTT GTG TGC CTG CTG AAT AAC
CTC GTC AAC TTT AGA CCT TGA CGG AGA CAA CAC ACG GAC GAC TTA TTG
Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn>

      490      500      510      520      530
      *      *      *      *      *
TTC TAT CCC AGA GAG GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC
AAG ATA GGG TCT CTC CGG TTT CAT GTC ACC TTC CAC CTA TTG CGG GAG
Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu>

      540      550      560      570
      *      *      *      *
CAA TCG GGT AAC TCC CAG GAG AGT GTC ACA GAG CAG GAC AGC AAG GAC
GTT AGC CCA TTG AGG GTC CTC TCA CAG TGT CTC GTC CTG TCG TTC CTG
Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp>

580      590      600      610      620
*      *      *      *      *
AGC ACC TAC AGC CTC AGC AGC ACC CTG ACG CTG AGC AAA GCA GAC TAC
TCG TGG ATG TCG GAG TCG TCG TGG GAC TGC GAC TCG TTT CGT CTG ATG
Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr>

      630      640      650      660      670
      *      *      *      *      *
GAG AAA CAC AAA GTC TAC GCC TGC GAA GTC ACC CAT CAG GGC CTG AGC
CTC TTT GTG TTT CAG ATG CCG ACG CTT CAG TGG GTA GTC CCG GAC TCG
Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser>

      680      690      700      710      720
      *      *      *      *      *
TCG CCC GTC ACA AAG AGC TTC AAC AGG GGA GAG TGT T AGA GGG AGA AGT
AGC GGG CAG TGT TTC TCG AAG TTG TCC CCT CTC ACA A TCT CCC TCT TCA
Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys>

      730      740      750      760      770
      *      *      *      *      *
GCC CCC ACC TGC TCC TCA GTT CCA GCC TGG GGA TCA TAA TCA GCC ATA
CGG GGG TGG ACG AGG AGT CAA GGT CCG ACC CCT AGT ATT AGT CGG TAT

      780      790      800      810
      *      *      *      *
CCA CAT TTG TAG AGG TTT TAC TTG CTT TAA AAA ACC TCC CAC ACC TCC
GGT GTA AAC ATC TCC AAA ATG AAC GAA ATT TTT TGG AGG GTC TGG AGG

820      830      840      850      860
*      *      *      *      *
CCC TGA ACC TGA AAC ATA AAA TGA ATG CAA TTG TTG TTG TTA ACT TGT
GGG ACT TGG ACT TTG TAT TTT ACT TAC GTT AAC AAC AAC AAT TGA ACA

      870      880      890      900      910
      *      *      *      *      *
TTA TTG CAG CTT ATA ATG GTT ACA AAT AAA GCA ATA GCA TCA CAA ATT
AAT AAC GTC GAA TAT TAC CAA TGT TTA TTT CGT TAT CGT AGT GTT TAA

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**FIG. 5 C**

```

      920      930      940      950      960
      *      *      *      *      *
TCA CAA ATA AAG CAT TTT TTT CAC TGC ATT CTA GTT GTG GTT TGT CCA
AGT GTT TAT TTC GTA AAA AAA GTG ACG TAA GAT CAA CAC CAA ACA GGT

      970      980      990      1000      1010
      *      *      *      *      *
AAC TCA TCA ATG TAT CTT ATC ATG TCT GGA TCC TCT ACG CCG GAC GCA
TTG AGT AGT TAC ATA GAA TAG TAC AGA CCT AGG AGA TGC GGC CTC CGT

      1020      1030      1040      1050
      *      *      *      *
TCG TGG CCG GCA TCA CCG GCG CCA CAG GTG CCG TTG CTG GCG CCT ATA
AGC ACC GGC CGT AGT GGC CGC GGT GTC CAC GCC AAC GAC CGC GGA TAT

1060      1070      1080      1090      1100
*      *      *      *      *
TCG CCG ACA TCA CCG ATG GGG AAG ATC GGG CTC GCC ACT TCG GGC TCA
AGC GGC TGT AGT GGC TAC CCC TTC TAG CCC GAG CCG TGA AGC CCG AGT

1110      1120      1130      1140      1150
*      *      *      *      *
TGA GCG CTT GTT TCG GCG TGG GTA TGG TGG CAG GCC CGT GGC CCG GGG
ACT CGC GAA CAA AGC CGC ACC CAT ACC ACC GTC CCG GCA CCG GCC CCC

      1160      1170      1180      1190      1200
      *      *      *      *      *
ACT GTT GGG CGC CAT CTC CTT GCA TGC ACC ATT CCT TGC GGC GGC GGT
TGA CAA CCC GCG GTA GAG GAA CGT ACG TGG TAA GGA ACG CCG CCG CCA

      1210      1220      1230      1240      1250
      *      *      *      *      *
GCT CAA CCG CCT CAA CCT ACT ACT GGG CTG CTT CCT AAT GCA GGA GTC
CGA GTT GCC GGA GTT GGA TGA TGA CCC GAC GAA GGA TTA CGT CCT CAG

      1260      1270      1280      1290
      *      *      *      *
GCA TAA GGG AGA GCG TCG ACC TCG GGC CGC GTT GCT GGC GTT TTT CCA
CGT ATT CCC TCT CGC AGC TGG AGC CCG GCG CAA CGA CCG CAA AAA GGT

1300      1310      1320      1330      1340
*      *      *      *      *
TAG GCT CCG CCC CCC TGA CGA GCA TCA CAA AAA TCG ACG CTC AAG TCA
ATC CGA GGC GGG GGG ACT GCT CGT AGT GTT TTT AGC TGC GAG TTC AGT

1350      1360      1370      1380      1390
*      *      *      *      *
GAG GTG GCG AAA CCC GAC AGG ACT ATA AAG ATA CCA GGC GTT TCC CCC
CTC CAC CGC TTT GGG CTG TCC TGA TAT TTC TAT GGT CCG CAA AGG GGG

      1400      1410      1420      1430      1440
      *      *      *      *      *
TGG AAG CTC CCT CGT GCG CTC TCC TGT TCC GAC CCT GCC GCT TAC CCG
ACC TTC GAG GGA GCA CGC GAG AGG ACA AGG CTG GGA CCG CGA ATG GCC

      1450      1460      1470      1480      1490
      *      *      *      *      *
ATA CCT GTC CGC CTT TCT CCC TTC GGG AAG CGT GGC GCT TTC TCA ATG
TAT GGA CAG GCG GAA AGA GGG AAG CCC TTC GCA CCG CGA AAG AGT TAC

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**FIG. 5 D**

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      1500      1510      1520      1530
      *        *        *        *
CTC ACG CTG TAG GTA TCT CAG TTC GGT GTA GGT CGT TCG CTC CAA GCT
GAG TGC GAC ATC CAT AGA GTC AAG CCA CAT CCA GCA AGC GAG GTT CGA

1540      1550      1560      1570      1580
*        *        *        *        *
GGG CTG TGT GCA CGA ACC CCC CGT TCA GCC CGA CCG CTG CGC CTT ATC
CCC GAC ACA CGT GCT TGG GGG GCA AGT CGG GCT GGC GAC GCG GAA TAG

1590      1600      1610      1620      1630
*        *        *        *        *
CGG TAA CTA TCG TCT TGA GTC CAA CCC GGT AAG ACA CGA CTT ATC GCC
GCC ATT GAT AGC AGA ACT CAG GTT GGG CCA TTC TGT GCT GAA TAG CGG

      1640      1650      1660      1670      1680
      *        *        *        *        *
ACT GGC AGC AGC CAC TGG TAA CAG GAT TAG CAG AGC GAG GTA TGT AGG
TGA CCG TCG TCG GTG ACC ATT GTC CTA ATC GTC TCG CTC CAT ACA TCC

      1690      1700      1710      1720      1730
      *        *        *        *        *
CGG TGC TAC AGA GTT CTT GAA GTG GTG GCC TAA CTA CGG CTA CAC TAG
GCC ACG ATG TCT CAA GAA CTT CAC CAC CGG ATT GAT GCC GAT GTG ATC

      1740      1750      1760      1770
      *        *        *        *
AAG GAC AGT ATT TGG TAT CTG CGC TCT GCT GAA GCC AGT TAC CTT CGG
TTC CTG TCA TAA ACC ATA GAC GCG AGA CGA CTT CGG TCA ATG GAA GCC

1780      1790      1800      1810      1820
*        *        *        *        *
AAA AAG AGT TGG TAG CTC TTG ATC CGG CAA ACA AAC CAC CGC TGG TAG
TTT TTC TCA ACC ATC GAG AAC TAG GCC GTT TGT TTG GTG GCG ACC ATC

1830      1840      1850      1860      1870
*        *        *        *        *
CGG TGG TTT TTT TGT TTG CAA GCA GCA GAT TAC GCG CAG AAA AAA AGG
GCC ACC AAA AAA ACA AAC GTT CGT CGT CTA ATG CGC GTC TTT TTT TCC

      1880      1890      1900      1910      1920
      *        *        *        *        *
ATC TCA AGA AGA TCC TTT GAT CTT TTC TAC GGG GTC TGA CGC TCA GTG
TAG AGT TCT TCT AGG AAA CTA GAA AAG ATG CCC CAG ACT GCG AGT CAC

      1930      1940      1950      1960      1970
      *        *        *        *        *
GAA CGA AAA CTC ACC TTA AGG GAT TTT GGT CAT GAG ATT ATC AAA AAG
CTT GCT TTT GAG TGC AAT TCC CTA AAA CCA GTA CTC TAA TAG TTT TTC

      1980      1990      2000      2010
      *        *        *        *
GAT CTT CAC CTA GAT CCT TTT AAA TTA AAA ATG AAG TTT TAA ATC AAT
CTA GAA GTG GAT CTA GGA AAA TTT AAT TTT TAC TTC AAA ATT TAG TTA

2020      2030      2040      2050      2060
*        *        *        *        *
CTA AAG TAT ATA TGA GTA AAC TTG GTC TGA CAG TTA CCA ATG CTT AAT
GAT TTC ATA TAT ACT CAT TTG AAC CAG ACT GTC AAT GGT TAC GAA TTA

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**FIG. 5 E**

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2070      2080      2090      2100      2110
*          *          *          *          *
CAG TGA GGC ACC TAT CTC AGC GAT CTG TCT ATT TCG TTC ATC CAT AGT
GTC ACT CCG TGG ATA GAG TCG CTA GAC AGA TAA AGC AAG TAG GTA TCA

2120      2130      2140      2150      2160
*          *          *          *          *
TGC CTG ACT CCC CGT CGT GTA GAT AAC TAC GAT ACG GGA GGG CTT ACC
ACG GAC TGA GGG GCA GCA CAT CTA TTG ATG CTA TGC CCT CCC GAA TGG

2170      2180      2190      2200      2210
*          *          *          *          *
ATC TGG CCC CAG TGC TGC AAT GAT ACC GCG AGA CCC ACG CTC ACC GGC
TAG ACC GGG GTC ACG ACG TTA CTA TGG CCG TCT GGG TGC CAG TGG CCG

2220      2230      2240      2250
*          *          *          *
TCC AGA TTT ATC AGC AAT AAA CCA GCC AGC CCG AAG GGC CGA GCG CAG
AGG TCT AAA TAG TCG TTA TTT GGT CCG TCG GCC TTC CCG GCT CGC GTC

2260      2270      2280      2290      2300
*          *          *          *          *
AAG TGG TCC TGC AAC TTT ATC CGC CTC CAT CCA GTC TAT TAA TTG TTG
TTC ACC AGG ACG TTG AAA TAG CCG GAG GTA GGT CAG ATA ATT AAC AAC

2310      2320      2330      2340      2350
*          *          *          *          *
CCG GGA AGC TAG AGT AAG TAG TTC GCC AGT TAA TAG TTT GCG CAA CGT
GGC CCT TCG ATC TCA TTC ATC AAG CCG TCA ATT ATC AAA CCG GTT GCA

2360      2370      2380      2390      2400
*          *          *          *          *
TGT TGC CAT TGC TAC AGG CAT CGT GGT GTC ACG CTC GTC GTT TGG TAT
ACA ACG GTA ACG ATG TCC GTA GCA CCA CAG TGC GAG CAG CAA ACC ATA

2410      2420      2430      2440      2450
*          *          *          *          *
GGC TTC ATT CAG CTC CCG TTC CCA ACG ATC AAG GCG AGT TAC ATG ATC
CCG AAG TAA GTC GAG GCC AAG GGT TGC TAG TTC CCG TCA ATG TAC TAG

2460      2470      2480      2490
*          *          *          *
CCC CAT GTT GTG CAA AAA AGC GGT TAG CTC CTT CCG TCC TCC GAT CGT
GGG GTA CAA CAC GTT TTT TCG CCA ATC GAG GAA GCC AGG AGG CTA GCA

2500      2510      2520      2530      2540
*          *          *          *          *
TGT CAG AAG TAA GTT GGC CCG AGT GTT ATC ACT CAT GGT TAT GGC AGC
ACA GTC TTC ATT CAA CCG GCG TCA CAA TAG TGA GTA CCA ATA CCG TCG

2550      2560      2570      2580      2590
*          *          *          *          *
ACT GCA TAA TTC TCT TAC TGT CAT GCC ATC CGT AAG ATG CTT TTC TGT
TGA CGT ATT AAG AGA ATG ACA GTA CCG TAG GCA TTC TAC GAA AAG ACA

2600      2610      2620      2630      2640
*          *          *          *          *
GAC TGG TGA GTA CTC AAC CAA GTC ATT CTG AGA ATA GTG TAT GCG GCG
CTG ACC ACT CAT GAG TTG GTT CAG TAA GAC TCT TAT CAC ATA CCG CCG

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**FIG. 5 F**

2650	2660	2670	2680	2690
ACC GAG TTG CTC TTG CCC GGC GTC AAC ACG GGA TAA TAC CGC GCC ACA				
TGG CTC AAC GAG AAC GGG CCG CAG TTG TGC CCT ATT ATG GCG CCG TGT				
2700	2710	2720	2730	
TAG CAG AAC TTT AAA AGT GCT CAT CAT TGG AAA ACG TTC TTC GGG GCG				
ATC GTC TTG AAA TTT TCA CGA GTA GTA ACC TTT TGC AAG AAG CCC CGC				
2740	2750	2760	2770	2780
AAA ACT CTC AAG GAT CTT ACC GCT GTT GAG ATC CAG TTC GAT GTA ACC				
TTT TGA GAG TTC CTA GAA TGG CGA CAA CTC TAG GTC AAG CTA CAT TGG				
2790	2800	2810	2820	2830
CAC TCG TGC ACC CAA CTG ATC TTC AGC ATC TTT TAC TTT CAC CAG CGT				
GTG AGC ACG TGG GTT GAC TAG AAG TCG TAG AAA ATG AAA GTG GTC GCA				
2840	2850	2860	2870	2880
TTC TGG GTG AGC AAA AAC AGG AAG GCA AAA TGC CGC AAA AAA GCG AAT				
AAG ACC CAC TCG TTT TTG TCC TTC CGT TTT ACG GCG TTT TTT CCC TTA				
2890	2900	2910	2920	2930
AAG GGC GAC ACG GAA ATG TTG AAT ACT CAT ACT CTT CCT TTT TCA ATA				
TTC CCG CTG TGC CTT TAC AAC TTA TGA GTA TGA GAA GGA AAA AGT TAT				
2940	2950	2960	2970	
TTA TTG AAG CAT TTA TCA GGG TTA TTG TCT CAT GAG CGG ATA CAT ATT				
AAT AAC TTC GTA AAT AGT CCC AAT AAC AGA GTA CTC GCC TAT GTA TAA				
2980	2990	3000	3010	3020
TGA ATG TAT TTA GAA AAA TAA ACA AAT AGG GGT TCC GCG CAC ATT TCC				
ACT TAC ATA AAT CTT TTT ATT TGT TTA TCC CCA AGG CGC GTG TAA AGG				
3030	3040	3050	3060	3070
CCG AAA AGT GCC ACC TGA CGT CTA AGA AAC CAT TAT TAT CAT GAC ATT				
GGC TTT TCA CGG TGG ACT GCA GAT TCT TTG GTA ATA ATA GTA CTG TAA				
3080	3090	3100	3110	3120
AAC CTA TAA AAA TAG GCG TAT CAC GAG GCC CTG ATG GCT CTT TGC GGC				
TTG GAT ATT TTT ATC CGC ATA GTG CTC CGG GAC TAC CGA GAA ACG CCG				
3130	3140	3150	3160	3170
ACC CAT CGT TCG TAA TGT TCC GTG GCA CCG AGG ACA ACC CTC AAG AGA				
TGG GTA GCA AGC ATT ACA AGG CAC CGT GGC TCC TGT TGG GAG TTC TCT				
3180	3190	3200	3210	
AAA TGT AAT CAC ACT GGC TCA CCT TCG GGT GGG CCT TTC TGC GTT TAT				
TTT ACA TTA GTG TGA CCG AGT GGA AGC CCA CCC GGA AAG ACG CAA ATA				

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**FIG. 5 G**

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3220      3230      3240      3250      3260
*          *          *          *          *
AAG GAG ACA CTT TAT GTT TAA GAA GGT TGG TAA ATT CCT TGC GGC TTT
TTC CTC TGT GAA ATA CAA ATT CTT CCA ACC ATT TAA GGA ACG CCG AAA

3270      3280      3290      3300      3310
*          *          *          *          *
GGC AGC CAA GCT AGA GAT CCG GCT GTG GAA TGT GTG TCA GTT AGG GTG
CCG TCG GTT CGA TCT CTA GGC CGA CAC CTT ACA CAC AGT CAA TCC CAC

3320      3330      3340      3350      3360
*          *          *          *          *
TGG AAA GTC CCC AGG CTC CCC AGC AGG CAG AAG TAT GCA AAG CAT GCA
ACC TTT CAG GGG TCC GAG GGG TCG TCC GTC TTC ATA CGT TTC GTA CGT

3370      3380      3390      3400      3410
*          *          *          *          *
TCT CAA TTA GTC AGC AAC CAG GCT CCC CAG CAG GCA GAA GTA TGC AAA
AGA GTT AAT CAG TCG TTG GTC CGA GGG GTC GTC CGT CTT CAT ACG TTT

3420      3430      3440      3450
*          *          *          *
GCA TGC ATC TCA ATT AGT CAG CAA CCA TAG TCC CGC CCC TAA CTC CGC
CGT ACG TAG AGT TAA TCA GTC GTT GGT ATC AGG GCG GGG ATT GAG GCG

3460      3470      3480      3490      3500
*          *          *          *          *
CCA TCC CGC CCC TAA CTC CGC CCA GTT CCG CCC ATT CTC CGC CCC ATG
GGT AGG GCG GGG ATT CAG GCG GGT CAA GGC GGG TAA GAG GCG GGG TAC

3510      3520      3530      3540      3550
*          *          *          *          *
GCT GAC TAA TTT TTT TTA TTT ATG CAG AGG CCG AGG CCG CCT CGG CCT
CGA CTG ATT AAA AAA AAT AAA TAC GTC TCC GGC TCC GGC GGA GCC GGA

3560      3570      3580      3590      3600
*          *          *          *          *
CTG AGC TAT TCC AGA AGT AGT GAG GAG GCT TTT TTG GAG GCC TAG GCT
GAC TCG ATA AGG TCT TCA TCA CTC CTC CGA AAA AAC CTC CCG ATC CGA

3610      3620      3630      3640      3650
*          *          *          *          *
TTT GCA AAA AGC TAG CTT GGG GCC ACC GCT CAG AGC ACC TTC CAC CAT
AAA CGT TTT TCG ATC GAA CCC CCG TGG CGA GTC TCG TGG AAG GTG GTA

3660      3670      3680      3690
*          *          *          *
GGC CAC CTC AGC AAG TTC CCA CTT GAA CAA AAA CAT CAA GCA AAT GTA
CCG GTG GAG TCG TTC AAG GGT GAA CTT GTT TTT GTA GTT CGT TTA CAT

3700      3710      3720      3730      3740
*          *          *          *          *
CTT GTG CCT GCC CCA GGG TGA GAA AGT CCA AGC CAT GTA TAT CTG GGT
GAA CAC GGA CCG GGT CCC ACT CTT TCA GGT TCG GTA CAT ATA GAC CCA

3750      3760      3770      3780      3790
*          *          *          *          *
TGA TCG TAC TCG AGA AGG ACT GCG CTG CAA AAC CCG CAC CCT GGA CTG
ACT ACC ATG ACC TCT TCC TGA CCG GAC GTT TTG GGC GTG GGA CCT GAC

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**FIG. 5 H**

3800 * TGA GCC CAA GTG TGT AGA AGA GTT ACC TGA GTG GAA TTT TGA TGG CTC ACT CGG GTT CAC ACA TCT TCT CAA TGG ACT CAC CTT AAA ACT ACC GAG	3810 * TGA GCC CAA GTG TGT AGA AGA GTT ACC TGA GTG GAA TTT TGA TGG CTC ACT CGG GTT CAC ACA TCT TCT CAA TGG ACT CAC CTT AAA ACT ACC GAG	3820 * TGA GCC CAA GTG TGT AGA AGA GTT ACC TGA GTG GAA TTT TGA TGG CTC ACT CGG GTT CAC ACA TCT TCT CAA TGG ACT CAC CTT AAA ACT ACC GAG	3830 * TGA GCC CAA GTG TGT AGA AGA GTT ACC TGA GTG GAA TTT TGA TGG CTC ACT CGG GTT CAC ACA TCT TCT CAA TGG ACT CAC CTT AAA ACT ACC GAG	3840 * TGA GCC CAA GTG TGT AGA AGA GTT ACC TGA GTG GAA TTT TGA TGG CTC ACT CGG GTT CAC ACA TCT TCT CAA TGG ACT CAC CTT AAA ACT ACC GAG
3850 * TAG TAC CTT TCA GTC TGA GGG CTC CAA CAG TGA CAT GTA TCT CAG CCC ATC ATG GAA AGT CAG ACT CCC GAG GTT GTC ACT GTA CAT AGA GTC GGG	3860 * TAG TAC CTT TCA GTC TGA GGG CTC CAA CAG TGA CAT GTA TCT CAG CCC ATC ATG GAA AGT CAG ACT CCC GAG GTT GTC ACT GTA CAT AGA GTC GGG	3870 * TAG TAC CTT TCA GTC TGA GGG CTC CAA CAG TGA CAT GTA TCT CAG CCC ATC ATG GAA AGT CAG ACT CCC GAG GTT GTC ACT GTA CAT AGA GTC GGG	3880 * TAG TAC CTT TCA GTC TGA GGG CTC CAA CAG TGA CAT GTA TCT CAG CCC ATC ATG GAA AGT CAG ACT CCC GAG GTT GTC ACT GTA CAT AGA GTC GGG	3890 * TAG TAC CTT TCA GTC TGA GGG CTC CAA CAG TGA CAT GTA TCT CAG CCC ATC ATG GAA AGT CAG ACT CCC GAG GTT GTC ACT GTA CAT AGA GTC GGG
3900 * TGT TGC CAT GTT TCG GGA CCC CTT CCG CAG AGA TCC CAA CAA GCT GGT ACA ACG GTA CAA AGC CCT GGG GAA GGC GTC TCT AGG GTT GTT CGA CCA	3910 * TGT TGC CAT GTT TCG GGA CCC CTT CCG CAG AGA TCC CAA CAA GCT GGT ACA ACG GTA CAA AGC CCT GGG GAA GGC GTC TCT AGG GTT GTT CGA CCA	3920 * TGT TGC CAT GTT TCG GGA CCC CTT CCG CAG AGA TCC CAA CAA GCT GGT ACA ACG GTA CAA AGC CCT GGG GAA GGC GTC TCT AGG GTT GTT CGA CCA	3930 * TGT TGC CAT GTT TCG GGA CCC CTT CCG CAG AGA TCC CAA CAA GCT GGT ACA ACG GTA CAA AGC CCT GGG GAA GGC GTC TCT AGG GTT GTT CGA CCA	
3940 * GTT CTG TGA AGT TTT CAA GTA CAA CCG GAA GCC TGC AGA GAC CAA TTT CAA GAC ACT TCA AAA GTT CAT GTT GGC CTT CCG ACG TCT CTG GTT AAA	3950 * GTT CTG TGA AGT TTT CAA GTA CAA CCG GAA GCC TGC AGA GAC CAA TTT CAA GAC ACT TCA AAA GTT CAT GTT GGC CTT CCG ACG TCT CTG GTT AAA	3960 * GTT CTG TGA AGT TTT CAA GTA CAA CCG GAA GCC TGC AGA GAC CAA TTT CAA GAC ACT TCA AAA GTT CAT GTT GGC CTT CCG ACG TCT CTG GTT AAA	3970 * GTT CTG TGA AGT TTT CAA GTA CAA CCG GAA GCC TGC AGA GAC CAA TTT CAA GAC ACT TCA AAA GTT CAT GTT GGC CTT CCG ACG TCT CTG GTT AAA	3980 * GTT CTG TGA AGT TTT CAA GTA CAA CCG GAA GCC TGC AGA GAC CAA TTT CAA GAC ACT TCA AAA GTT CAT GTT GGC CTT CCG ACG TCT CTG GTT AAA
3990 * AAG GCA CTC GTG TAA ACG GAT AAT GGA CAT GGT GAG CAA CCA GCA CCC TTC CGT GAG CAC ATT TGC CTA TTA CCT GTA CCA CTC GTT GGT CGT GGG	4000 * AAG GCA CTC GTG TAA ACG GAT AAT GGA CAT GGT GAG CAA CCA GCA CCC TTC CGT GAG CAC ATT TGC CTA TTA CCT GTA CCA CTC GTT GGT CGT GGG	4010 * AAG GCA CTC GTG TAA ACG GAT AAT GGA CAT GGT GAG CAA CCA GCA CCC TTC CGT GAG CAC ATT TGC CTA TTA CCT GTA CCA CTC GTT GGT CGT GGG	4020 * AAG GCA CTC GTG TAA ACG GAT AAT GGA CAT GGT GAG CAA CCA GCA CCC TTC CGT GAG CAC ATT TGC CTA TTA CCT GTA CCA CTC GTT GGT CGT GGG	4030 * AAG GCA CTC GTG TAA ACG GAT AAT GGA CAT GGT GAG CAA CCA GCA CCC TTC CGT GAG CAC ATT TGC CTA TTA CCT GTA CCA CTC GTT GGT CGT GGG
4040 * CTG GTT TGG AAT GGA ACA GGA GTA TAC TCT GAT GGG AAC AGA TGG GCA GAC CAA ACC TTA CCT TGT CCT CAT ATG AGA CTA CCC TTG TCT ACC CGT	4050 * CTG GTT TGG AAT GGA ACA GGA GTA TAC TCT GAT GGG AAC AGA TGG GCA GAC CAA ACC TTA CCT TGT CCT CAT ATG AGA CTA CCC TTG TCT ACC CGT	4060 * CTG GTT TGG AAT GGA ACA GGA GTA TAC TCT GAT GGG AAC AGA TGG GCA GAC CAA ACC TTA CCT TGT CCT CAT ATG AGA CTA CCC TTG TCT ACC CGT	4070 * CTG GTT TGG AAT GGA ACA GGA GTA TAC TCT GAT GGG AAC AGA TGG GCA GAC CAA ACC TTA CCT TGT CCT CAT ATG AGA CTA CCC TTG TCT ACC CGT	4080 * CTG GTT TGG AAT GGA ACA GGA GTA TAC TCT GAT GGG AAC AGA TGG GCA GAC CAA ACC TTA CCT TGT CCT CAT ATG AGA CTA CCC TTG TCT ACC CGT
4090 * CCC TTT TGG TTG GCC TTC CAA TGG CTT TCC TGG GCC CCA AGG TCC GTA GGG AAA ACC AAC CCG AAG GTT ACC GAA AGG ACC CCG GGT TCC AGG CAT	4100 * CCC TTT TGG TTG GCC TTC CAA TGG CTT TCC TGG GCC CCA AGG TCC GTA GGG AAA ACC AAC CCG AAG GTT ACC GAA AGG ACC CCG GGT TCC AGG CAT	4110 * CCC TTT TGG TTG GCC TTC CAA TGG CTT TCC TGG GCC CCA AGG TCC GTA GGG AAA ACC AAC CCG AAG GTT ACC GAA AGG ACC CCG GGT TCC AGG CAT	4120 * CCC TTT TGG TTG GCC TTC CAA TGG CTT TCC TGG GCC CCA AGG TCC GTA GGG AAA ACC AAC CCG AAG GTT ACC GAA AGG ACC CCG GGT TCC AGG CAT	4130 * CCC TTT TGG TTG GCC TTC CAA TGG CTT TCC TGG GCC CCA AGG TCC GTA GGG AAA ACC AAC CCG AAG GTT ACC GAA AGG ACC CCG GGT TCC AGG CAT
4140 * TTA CTG TGG TGT GGG CCG AGA CAA AGC CTA TCG CAG GGA TAT CGT GGA AAT GAC ACC ACA CCC GCG TCT GTT TCG GAT ACC GTC CCT ATA GCA CCT	4150 * TTA CTG TGG TGT GGG CCG AGA CAA AGC CTA TCG CAG GGA TAT CGT GGA AAT GAC ACC ACA CCC GCG TCT GTT TCG GAT ACC GTC CCT ATA GCA CCT	4160 * TTA CTG TGG TGT GGG CCG AGA CAA AGC CTA TCG CAG GGA TAT CGT GGA AAT GAC ACC ACA CCC GCG TCT GTT TCG GAT ACC GTC CCT ATA GCA CCT	4170 * TTA CTG TGG TGT GGG CCG AGA CAA AGC CTA TCG CAG GGA TAT CGT GGA AAT GAC ACC ACA CCC GCG TCT GTT TCG GAT ACC GTC CCT ATA GCA CCT	
4180 * GGC TCA CTA CCG CCG CTG CTT GTA TGC TGG GGT CAA GAT TAC AGG AAC CCG AGT GAT GGC CCG GAC GAA CAT ACC ACC CCA GTT CTA ATG TCC TTG	4190 * GGC TCA CTA CCG CCG CTG CTT GTA TGC TGG GGT CAA GAT TAC AGG AAC CCG AGT GAT GGC CCG GAC GAA CAT ACC ACC CCA GTT CTA ATG TCC TTG	4200 * GGC TCA CTA CCG CCG CTG CTT GTA TGC TGG GGT CAA GAT TAC AGG AAC CCG AGT GAT GGC CCG GAC GAA CAT ACC ACC CCA GTT CTA ATG TCC TTG	4210 * GGC TCA CTA CCG CCG CTG CTT GTA TGC TGG GGT CAA GAT TAC AGG AAC CCG AGT GAT GGC CCG GAC GAA CAT ACC ACC CCA GTT CTA ATG TCC TTG	4220 * GGC TCA CTA CCG CCG CTG CTT GTA TGC TGG GGT CAA GAT TAC AGG AAC CCG AGT GAT GGC CCG GAC GAA CAT ACC ACC CCA GTT CTA ATG TCC TTG
4230 * AAA TGC TCA GGT CAT GGC TCC CCA GTG GGA ACT CCA AAT AGG ACC CTG TTT ACG ACT CCA GTA CCG ACG GGT CAC CCT TGA GGT TTA TCC TGG GAC	4240 * AAA TGC TCA GGT CAT GGC TCC CCA GTG GGA ACT CCA AAT AGG ACC CTG TTT ACG ACT CCA GTA CCG ACG GGT CAC CCT TGA GGT TTA TCC TGG GAC	4250 * AAA TGC TCA GGT CAT GGC TCC CCA GTG GGA ACT CCA AAT AGG ACC CTG TTT ACG ACT CCA GTA CCG ACG GGT CAC CCT TGA GGT TTA TCC TGG GAC	4260 * AAA TGC TCA GGT CAT GGC TCC CCA GTG GGA ACT CCA AAT AGG ACC CTG TTT ACG ACT CCA GTA CCG ACG GGT CAC CCT TGA GGT TTA TCC TGG GAC	4270 * AAA TGC TCA GGT CAT GGC TCC CCA GTG GGA ACT CCA AAT AGG ACC CTG TTT ACG ACT CCA GTA CCG ACG GGT CAC CCT TGA GGT TTA TCC TGG GAC
4280 * TGA AGG AAT CCG CAT GGG AGA TCA TCT CTG GGT GGC CCG TTT CAT CTT ACT TCC TTA GGC GTA CCC TCT AGT AGA GAC CCA CCG GGC AAA GTA GAA	4290 * TGA AGG AAT CCG CAT GGG AGA TCA TCT CTG GGT GGC CCG TTT CAT CTT ACT TCC TTA GGC GTA CCC TCT AGT AGA GAC CCA CCG GGC AAA GTA GAA	4300 * TGA AGG AAT CCG CAT GGG AGA TCA TCT CTG GGT GGC CCG TTT CAT CTT ACT TCC TTA GGC GTA CCC TCT AGT AGA GAC CCA CCG GGC AAA GTA GAA	4310 * TGA AGG AAT CCG CAT GGG AGA TCA TCT CTG GGT GGC CCG TTT CAT CTT ACT TCC TTA GGC GTA CCC TCT AGT AGA GAC CCA CCG GGC AAA GTA GAA	4320 * TGA AGG AAT CCG CAT GGG AGA TCA TCT CTG GGT GGC CCG TTT CAT CTT ACT TCC TTA GGC GTA CCC TCT AGT AGA GAC CCA CCG GGC AAA GTA GAA
4330 * NCA TCG AGT ATG TGA AGA CTT TGG GGT AAT AGC AAC CTT TGA CCC CAA NGT AGC TCA TAC ACT TCT GAA ACC CCA TTA TCG TTG GAA ACT GGG GTT	4340 * NCA TCG AGT ATG TGA AGA CTT TGG GGT AAT AGC AAC CTT TGA CCC CAA NGT AGC TCA TAC ACT TCT GAA ACC CCA TTA TCG TTG GAA ACT GGG GTT	4350 * NCA TCG AGT ATG TGA AGA CTT TGG GGT AAT AGC AAC CTT TGA CCC CAA NGT AGC TCA TAC ACT TCT GAA ACC CCA TTA TCG TTG GAA ACT GGG GTT	4360 * NCA TCG AGT ATG TGA AGA CTT TGG GGT AAT AGC AAC CTT TGA CCC CAA NGT AGC TCA TAC ACT TCT GAA ACC CCA TTA TCG TTG GAA ACT GGG GTT	4370 * NCA TCG AGT ATG TGA AGA CTT TGG GGT AAT AGC AAC CTT TGA CCC CAA NGT AGC TCA TAC ACT TCT GAA ACC CCA TTA TCG TTG GAA ACT GGG GTT

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**FIG. 5 I**

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      4380      4390      4400      4410
      *        *        *        *
GCC CAT TCC TGG GAA CTG GAA TGG TGC AGG CTG CCA TAC CAA CTT TAG
CGG GTA AGG ACC CTT GAC CTT ACC ACG TCC GAC GGT ATG GTT GAA ATC

4420      4430      4440      4450      4460
      *        *        *        *
CAC CAA GGC CAT GCG GGA GGA GAA TGG TCT GAA GCA CAT CGA GGA GGC
GTG GTT CCG GTA CGC CCT CCT CTT ACC AGA CTT CGT GTA GCT CCT CCG

4470      4480      4490      4500      4510
      *        *        *        *
CAT CGA GAA ACT AAG CAA GCG GCA CCG GTA CCA CAT TCG AGC CTA CGA
GTA GCT CTT TGA TTC GTT CGC CGT GGC CAT GGT GTA AGC TCG GAT GCT

4520      4530      4540      4550      4560
      *        *        *        *
TCC CAA GGG GGG CCT GGA CAA TGC CCG TGG TCT GAC TGG GTT CCA CGA
AGG GTT CCC CCC GGA CCT GTT ACG GGC ACC AGA CTG ACC CAA GGT GCT

4570      4580      4590      4600      4610
      *        *        *        *
AAC GTC CAA CAT CAA CGA CTT TTC TGC TGG TGT CCG CAA TCG CAG TGC
TTG CAG GTT GTA GTT GCT GAA AAG ACG ACC ACA GCG GTT AGC GTC ACG

4620      4630      4640      4650
      *        *        *        *
CAG CAT CCG CAT TCC CCG GAC TGT CCG CCA GGA GAA AGG TTA CTT
GTC GTA GGC GTA AGG GGC CTG ACA GCC GGT CCT CTT CTT TCC AAT GAA

4660      4670      4680      4690      4700
      *        *        *        *
TGA AGA CCG CCG CCC CTC TGC CAA TTG TGA CCC CTT TGC AGT GAC AGA
ACT TCT GGC GCC GGG GAG ACG GTT AAC ACT GGG GAA ACG TCA CTG TCT

4710      4720      4730      4740      4750
      *        *        *        *
AGC CAT CGT CCG CAC ATG CCT TCT CAA TGA GAC TGG CCA CGA GCC CTT
TCG GTA GCA GGC GTG TAC GGA AGA GTT ACT CTG ACC GGT GCT CCG GAA

4760      4770      4780      4790      4800
      *        *        *        *
CCA ATA CAA AAA CTA ATT AGA CTT TGA GTG ATC TTG AGC CTT TCC TAG
GGT TAT GTT TTT GAT TAA TCT GAA ACT CAC TAG AAC TCG GAA AGG ATC

4810      4820      4830      4840      4850
      *        *        *        *
TTC ATC CCA CCC CCG CCC AGA GAG ATC TTT GTG AAG GAA CCT TAC TTC
AAG TAG GGT GGG GCG GGG TCT CTC TAG AAA CAC TTC CTT GGA ATG AAG

4860      4870      4880      4890
      *        *        *        *
TGT GGT GTG ACA TAA TTG GAC AAA CTA CCT ACA GAG ATT TAA AGC TCT
ACA CCA CAC TGT ATT AAC CTG TTT GAT GGA TGT CTC TAA ATT TCG AGA

4900      4910      4920      4930      4940
      *        *        *        *
AAG GTA AAT ATA AAA TTT TTA AGT GTA TAA TGT GTT AAA CTA CTG ATT
TTC CAT TTA TAT TTT AAA AAT TCA CAT ATT ACA CAA TTT GAT GAC TAA

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**FIG. 5 J**

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4950      4960      4970      4980      4990
*          *          *          *          *
CTA ATT GTT TGT GTA TTT TAG ATT CCA ACC TAT GGA ACT GAT GAA TGG
GAT TAA CAA ACA CAT AAA ATC TAA GGT TGG ATA CCT TGA CTA CTT ACC

5000      5010      5020      5030      5040
*          *          *          *          *
GAG CAG TGG TGG AAT GCC TTT AAT GAG GAA AAC CTG TTT TGC TCA GAA
CTC GTC ACC ACC TTA CGG AAA TTA CTC CTT TTG GAC AAA ACG AGT CTT

5050      5060      5070      5080      5090
*          *          *          *          *
GAA ATG CCA TCT AGT GAT GAT GAG GCT ACT GCT GAC TCT CAA CAT TCT
CTT TAC GGT AGA TCA CTA CTA CTC CGA TGA CGA CTG AGA GTT GTA AGA

5100      5110      5120      5130
*          *          *          *
ACT CCT CCA AAA AAG AAG AGA AAG GTA GAA GAC CCC AAG GAC TTT CCT
TGA GGA GGT TTT TTC TTC TCT TTC CAT CTT CTG GGG TTC CTG AAA GGA

5140      5150      5160      5170      5180
*          *          *          *          *
TCA GAA TTG CTA AGT TTT TTG AGT CAT GCT GTG TTT AGT AAT AGA ACT
AGT CTT AAC GAT TCA AAA AAC TCA GTA CGA CAC AAA TCA TTA TCT TGA

5190      5200      5210      5220      5230
*          *          *          *          *
CTT GCT TGC TTT GCT ATT TAC ACC ACA AAG GAA AAA GCT GCA CTG CTA
GAA CGA ACG AAA CGA TAA ATG TGG TGT TTC CTT TTT CGA CGT GAC GAT

5240      5250      5260      5270      5280
*          *          *          *          *
TAC AAG AAA ATT ATG GAA AAA TAT TCT GTA ACC TTT ATA AGT AGC CAT
ATG TTC TTT TAA TAC CTT TTT ATA AGA CAT TGG AAA TAT TCA TCC GTA

5290      5300      5310      5320      5330
*          *          *          *          *
AAC AGT TAT AAT CAT AAC ATA CTG TTT TTT CTT ACT CCA CAC AGG CAT
TTG TCA ATA TTA GTA TTG TAT GAC AAA AAA GAA TGA GGT GTG TCC GTA

5340      5350      5360      5370
*          *          *          *
AGA GTG TCT GCT ATT AAT AAC TAT GCT CAA AAA TTG TGT ACC TTT AGC
TCT CAC AGA CGA TAA TTA TTG ATA CGA GTT TTT AAC ACA TGG AAA TCG

5380      5390      5400      5410      5420
*          *          *          *          *
TTT TTA ATT TGT AAA GGG GTT AAT AAG GAA TAT TTG ATG TAT AGT GCC
AAA AAT TAA ACA TTT CCC CAA TTA TTC CTT ATA AAC TAC ATA TCA CGG

5430      5440      5450      5460      5470
*          *          *          *          *
TTG ACT AGA GAT CAT AAT CAG CCA TAC CAC ATT TGT AGA GGT TTT ACT
AAC TGA TCT CTA GTA TTA GTC GGT ATG GTG TAA ACA TCT CCA AAA TGA

5480      5490      5500      5510      5520
*          *          *          *          *
TGC TTT AAA AAA CCT CCC ACA CCT CCC CCT GAA CCT GAA ACA TAA AAT
ACG AAA TTT TTT GGA GGG TGT GGA GGG GGA CTT GGA CTT TGT ATT TTA

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**FIG. 5 K**

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      5530      5540      5550      5560      5570
      *        *        *        *        *
GAA TGC AAT TGT TGT TGT TAA CTT GTT TAT TGC AGC TTA TAA TGG TTA
CTT ACG TTA ACA ACA ACA ATT GAA CAA ATA ACG TCG AAT ATT ACC AAT

      5580      5590      5600      5610
      *        *        *        *
CAA ATA AAG CAA TAG CAT CAC AAA TTT CAC AAA TAA AGC ATT TTT TTC
GTT TAT TTC GTT ATC GTA GTG TTT AAA GTG TTT ATT TCG TAA AAA AAG

5620      5630      5640      5650      5660
*        *        *        *        *
ACT GCA TTC TAG TTG TGG TTT GTC CAA ACT CAT CAA TGT ATC TTA TCA
TGA CGT AAG ATC AAC ACC AAA CAG GTT TGA GTA GTT ACA TAG AAT AGT

5670      5680      5690      5700      5710
*        *        *        *        *
TGT CTG GAT CTC TAG CTT CGT GTC AAG GAC GGT GAC TGC AGT GAA TAA
ACA GAC CTA GAG ATC GAA GCA CAG TTC CTG CCA CTG ACG TCA CTT ATT

      5720      5730      5740      5750      5760
      *        *        *        *        *
TAA AAT GTG TGT TTG TCC GAA ATA CGC GTT TTG AGA TTT CTG TCG CCG
ATT TTA CAC ACA AAC AGG CTT TAT GCG CAA AAC TCT AAA GAC AGC GGC

      5770      5780      5790      5800      5810
      *        *        *        *        *
ACT AAA TTC ATG TCG CGC GAT AGT GGT GTT TAT CGC CGA TAG AGA TGG
TGA TTT AAG TAC AGC GCG CTA TCA CCA CAA ATA GCG GCT ATC TCT ACC

      5820      5830      5840      5850
      *        *        *        *
CGA TAT TGG AAA AAT CGA TAT TTG AAA ATA TCG CAT ATT GAA AAT GTC
GCT ATA ACC TTT TTA GCT ATA AAC TTT TAT ACC GTA TAA CTT TTA CAG

5860      5870      5880      5890      5900
*        *        *        *        *
GCC GAT GTG AGT TTC TGT GTA ACT GAT ATC GCC ATT TTT CCA AAA GTG
CGG CTA CAC TCA AAG ACA CAT TGA CTA TAG CCG TAA AAA GGT TTT CAC

5910      5920      5930      5940      5950
*        *        *        *        *
ATT TTT GGG CAT ACG CGA TAT CTG GCG ATA GCG CTT ATA TCG TTT ACG
TAA AAA CCC GTA TGC GCT ATA GAC CCG TAT CCG GAA TAT AGC AAA TGC

      5960      5970      5980      5990      6000
      *        *        *        *        *
GGG GAT GGC GAT AGA CGA CTT TGG TGA CTT GGG CGA TTC TGT GTG TCG
CCC CTA CCG CTA TCT GCT GAA ACC ACT GAA CCC GCT AAG ACA CAC AGC

      6010      6020      6030      6040      6050
      *        *        *        *        *
CAA ATA TCG CAG TTT CGA TAT AGG TGA CAG ACC ATA TGA GGC TAT ATC
GTT TAT AGC GTC AAA GCT ATA TCC ACT GTC TGC TAT ACT CCG ATA TAG

      6060      6070      6080      6090
      *        *        *        *
GCC GAT AGA GGC GAC ATC AAG CTG GCA CAT GGC CAA TGC ATA TCG ATC
CGG CTA TCT CCG CTG TAG TTC GAC CGT GTA CCG GTT ACG TAT AGC TAG

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**FIG. 5 L**

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6100      6110      6120      6130      6140
*         *         *         *         *
TAT ACA TTG AAT CAA TAT TGG CCA TTA GCC ATA TTA TTC ATT GGT TAT
ATA TGT AAC TTA GTT ATA ACC GGT AAT CCG TAT AAT AAG TAA CCA ATA

6150      6160      6170      6180      6190
*         *         *         *         *
ATA GCA TAA ATC AAT ATT GGC TAT TGG CCA TTG CAT ACG TTG TAT CCA
TAT CGT ATT TAG TTA TAA CCG ATA ACC GGT AAC GTA TGC AAC ATA GGT

6200      6210      6220      6230      6240
*         *         *         *         *
TAT CAT AAT ATG TAC ATT TAT ATT GGC TCA TGT CCA ACA TTA CCG CCA
ATA GTA TTA TAC ATG TAA ATA TAA CCG AGT ACA GGT TGT AAT GGC GGT

6250      6260      6270      6280      6290
*         *         *         *         *
TGT TGA CAT TGA TTA TTG ACT AGT TAT TAA TAG TAA TCA ATT ACG GGG
ACA ACT GTA ACT AAT AAC TGA TCA ATA ATT ATC ATT AGT TAA TGC CCC

6300      6310      6320      6330
*         *         *         *
TCA TTA GTT CAT AGC CCA TAT ATG GAG TTC CCG GTT ACA TAA CTT ACG
AGT AAT CAA GTA TCG GGT ATA TAC CTC AAG GCG CAA TGT ATT GAA TGC

6340      6350      6360      6370      6380
*         *         *         *         *
GTA AAT GGC CCG CCT GGC TGA CCG CCC AAC GAC CCC CCG CCA TTG ACG
CAT TTA CCG GGC GGA CCG ACT GGC GGG TTG CTG GGG GCG GGT AAC TGC

6390      6400      6410      6420      6430
*         *         *         *         *
TCA ATA ATG ACG TAT GTT CCC ATA GTA ACG CCA ATA GGG ACT TTC CAT
AGT TAT TAC TGC ATA CAA GGG TAT CAT TGC GGT TAT CCC TGA AAG GTA

6440      6450      6460      6470      6480
*         *         *         *         *
TGA CGT CAA TGG GTG GAG TAT TTA CCG TAA ACT GCC CAC TTG GCA GTA
ACT GCA GTT ACC CAC CTC ATA AAT GCC ATT TGA CCG GTG AAC CGT CAT

6490      6500      6510      6520      6530
*         *         *         *         *
CAT CAA GTG TAT CAT ATG CCA AGT ACG CCC CCT ATT GAC GTC AAT GAC
GTA GTT CAC ATA GTA TAC GGT TCA TGC GGG GGA TAA CTG CAG TTA CTG

6540      6550      6560      6570
*         *         *         *
GGT AAA TGG CCC GCC TGG CAT TAT GCC CAG TAC ATG ACC TTA TGG GAC
CCA TTT ACC GGG CCG ACC GTA ATA CCG GTC ATG TAC TGG AAT ACC CTG

6580      6590      6600      6610      6620
*         *         *         *         *
TTT CCT ACT TGG CAG TAC ATC TAC GTA TTA GTC ATC GCT ATT ACC ATG
AAA GGA TGA ACC GTC ATG TAG ATG CAT AAT CAG TAG CGA TAA TGG TAC

6630      6640      6650      6660      6670
*         *         *         *         *
GTG ATG CCG TTT TGG CAG TAC ATC AAT GGG CGT GGA TAG CCG TTT GAC
CAC TAC GCC AAA ACC GTC ATG TAG TTA CCC GCA CCT ATC GCC AAA CTG

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**FIG. 5 M**

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        6680          6690          6700          6710          6720
        *           *           *           *           *
TCA CGG GGA TTT CCA AGT CTC CAC CCC ATT GAC GTC AAT GGG AGT TTG
AGT GCC CCT AAA GGT TCA GAG GTG GGG TAA CTG CAG TTA CCC TCA AAC

        6730          6740          6750          6760          6770
        *           *           *           *           *
TTT TGG CAC CAA AAT CAA CGG GAC TTT CCA AAA TGT CGT AAC AAC TCC
AAA ACC GTG GTT TTA GTT GCC CTG AAA GGT TTT ACA GCA TTG TTG AGG

        6780          6790          6800          6810
        *           *           *           *
GCC CCA TTG ACG CAA ATG GGC GGT AGG CGT GTA CCG TGG GAG GTC TAT
CGG GGT AAC TGC GTT TAC CCG CCA TCC GCA CAT GCC ACC CTC CAG ATA

6820          6830          6840          6850          6860
*           *           *           *           *
ATA AGC AGA GCT CGT TTA GTG AAC CGT CAG ATC GCC TGG AGA CGC CAT
TAT TCG TCT CGA GCA AAT CAC TTG GCA GTC TAG CCG ACC TCT GCG GTA

6870          6880          6890          6900          6910
*           *           *           *           *
CCA CGC TGT TTT GAC CTC CAT AGA AGA CAC CGG GAC CGA TCC AGC CTC
GGT GCG ACA AAA CTG GAG GTA TCT TCT GTG GCC CTG GCT AGG TCG GAG

        6920          6930          6940          6950          6960
        *           *           *           *           *
CGC GGC CGG GAA CCG TGC ATT GGA ACG CCG ATT CCC CGT GCC AAG AGT
GCG CCG GCC CTT GCC ACG TAA CCT TGC GCC TAA GGG GCA CCG TTC TCA

        6970          6980          6990          7000          7010
        *           *           *           *           *
GAC GTA AGT ACC GCC TAT AGA GTC TAT AGG CCC ACC CCC TTG GCT TCT
CTG CAT TCA TGG CCG ATA TCT CAG ATA TCC GGG TGG GGG AAC CGA AGA

        7020          7030          7040          7050
        *           *           *           *
TAT GCA TGC TAT ACT GTT TTT GGC TTG GGG TCT ATA CAC CCC CGC TTC
ATA CGT ACG ATA TGA CAA AAA CCG AAC CCC AGA TAT GTG GGG GCG AAG

7060          7070          7080          7090          7100
*           *           *           *           *
CTC ATG TTA TAG GTG ATG GTA TAG CTT AGC CTA TAG GTG TGG GTT ATT
GAG TAC AAT ATC CAC TAC CAT ATC GAA TCG GAT ATC CAC ACC CAA TAA

7110          7120          7130          7140          7150
*           *           *           *           *
GAC CAT TAT TGA CCA CTC CCC TAT TGG TGA CGA TAC TTT CCA TTA CTA
CTG GTA ATA ACT GGT GAG GGG ATA ACC ACT GCT ATG AAA GGT AAT GAT

        7160          7170          7180          7190          7200
        *           *           *           *           *
ATC CAT AAC ATG GCT CTT TGC CAC AAC TCT CTT TAT TGG CTA TAT GCC
TAG GTA TTG TAC CGA GAA ACG GTG TTG AGA GAA ATA ACC GAT ATA CCG

        7210          7220          7230          7240          7250
        *           *           *           *           *
AAT ACA CTG TCC TTC AGA GAC TGA CAC GGA CTC TGT ATT TTT ACA GGA
TTA TGT GAC AGG AAG TCT CTG ACT GTG CCT GAG ACA TAA AAA TGT CCT

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RECTIFIED SHEET (RULE 91)

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**FIG. 5 N**

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      7260      7270      7280      7290
      *      *      *      *
TGG GGT CTC ATT TAT TAT TTA CAA ATT CAC ATA TAC AAC ACC ACC GTC
ACC CCA GAG TAA ATA ATA AAT GTT TAA GTG TAT ATG TTG TGG TGG CAG

7300      7310      7320      7330      7340
      *      *      *      *      *
CCC AGT GCC CGC AGT TTT TAT TAA ACA TAA CGT GGG ATC TCC ACG CGA
GGG TCA CGG GCG TCA AAA ATA ATT TGT ATT GCA CCC TAG AGG TGC GCT

7350      7360      7370      7380      7390
      *      *      *      *      *
ATC TCG GGT ACG TGT TCC GGA CAT GGG CTC TTC TCC GGT AGC GGC GGA
TAG AGC CCA TGC ACA AGG CCT GTA CCC GAG AAG AGG CCA TCG CCG CCT

7400      7410      7420      7430      7440
      *      *      *      *      *
GCT TCT ACA TCC GAG CCC TGC TCC CAT GCC TCC AGC GAC TCA TGG TCG
CGA AGA TGT AGG CTC GGG ACG AGG GTA CCG AGG TCG CTG AGT ACC AGC

7450      7460      7470      7480      7490
      *      *      *      *      *
CTC GGC AGC TCC TTG CTC CTA ACA GTG GAG GCC AGA CTT AGG CAC AGC
GAG CCG TCG AGG AAC GAG GAT TGT CAC CTC CCG TCT GAA TCC GTG TCG

7500      7510      7520      7530
      *      *      *      *
ACG ATG CCC ACC ACC ACC AGT GTG CCG CAC AAG GCC GTG GCG GTA GGG
TGC TAC GGG TGG TGG TGG TCA CAC GGC GTG TTC CCG CAC CCG CAT CCC

7540      7550      7560      7570      7580
      *      *      *      *      *
TAT GTG TCT GAA AAT GAG CTC GCG GAG CCG GCT TGC ACC GCT GAC GCA
ATA CAC AGA CTT TTA CTC GAG CCC CTC GCC CGA ACC TGG CGA CTG CGT

7590      7600      7610      7620      7630
      *      *      *      *      *
TTT GGA AGA CTT AAG GCA GCG GCA GAA GAA GAT GCA GGC AGC TGA GTT
AAA CCT TCT GAA TTC CGT CCG CGT CTT CTT CTA CGT CCG TCG ACT CAA

7640      7650      7660      7670      7680
      *      *      *      *      *
GTT GTG TTC TGA TAA GAG TCA GAG GTA ACT CCC GTT CCG GTG CTG TTA
CAA CAC AAG ACT ATT CTC AGT CTC CAT TGA GCG CAA CCG CAC GAC AAT

7690      7700      7710      7720      7730
      *      *      *      *      *
ACG GTG GAG GGC AGT GTA CTC TGA GCA GTA CTC GTT GCT GCC GCG CCG
TGC CAC CTC CCG TCA CAT CAG ACT CGT CAT GAG CAA CGA CCG CCG GCG

7740      7750      7760      7770
      *      *      *      *
GCC ACC AGA CAT AAT AGC TGA CAG ACT AAC AGA CTC TTC CTT TCC ATG
CGG TGG TCT GTA TTA TCG ACT GTC TGA TTG TCT GAC AAG GAA AGG TAC

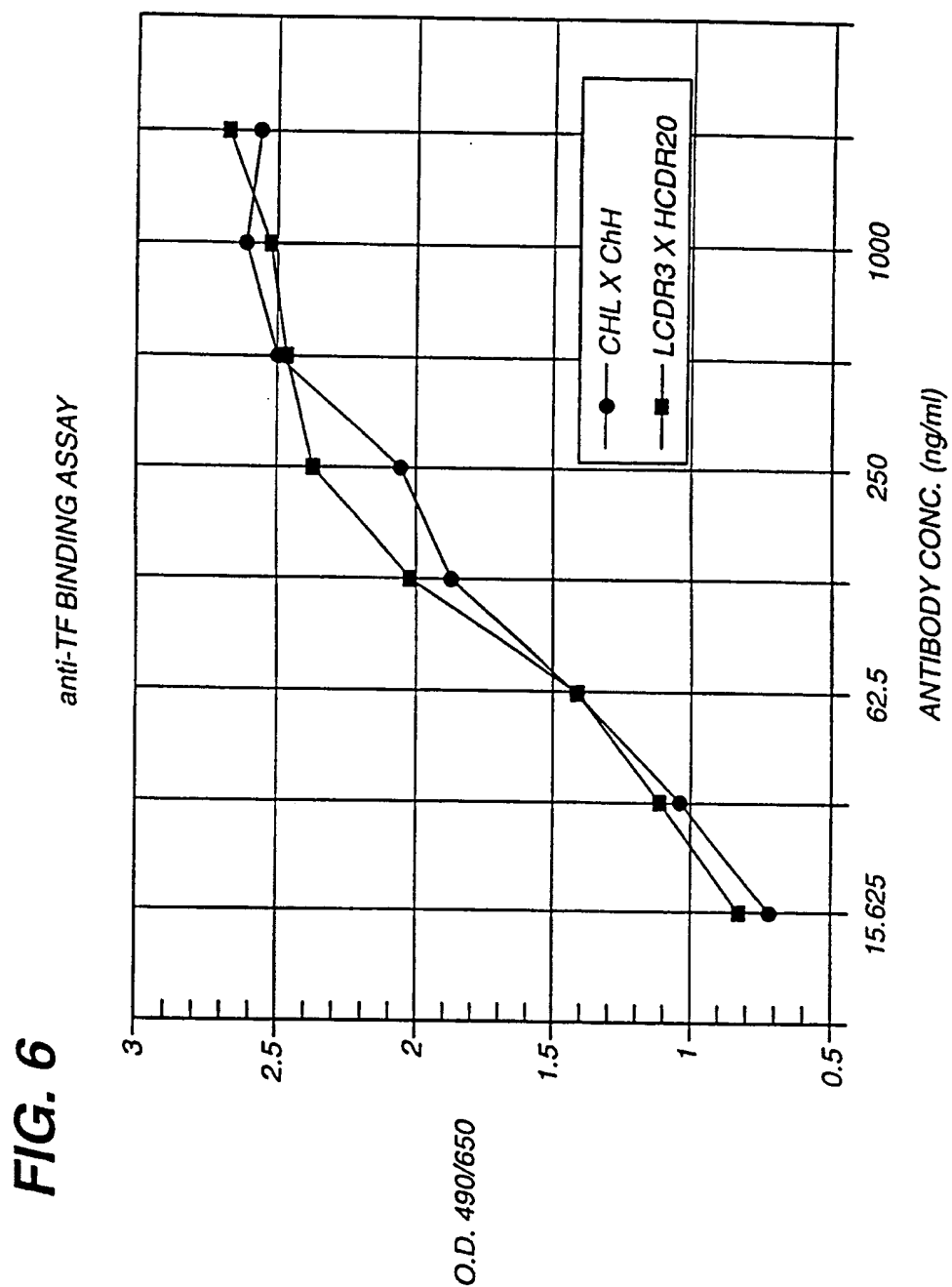
7780      7790      7800      7810      7820
      *      *      *      *      *
GGT CTT TTC TGC AGT CAC CGT CCT TGA CAC GAA GCT TGG GCT GCA GGT
CCA GAA AAG ACG TCA GTG CCA GGA ACT GTG CTT CGA ACC CGA CGT CCA

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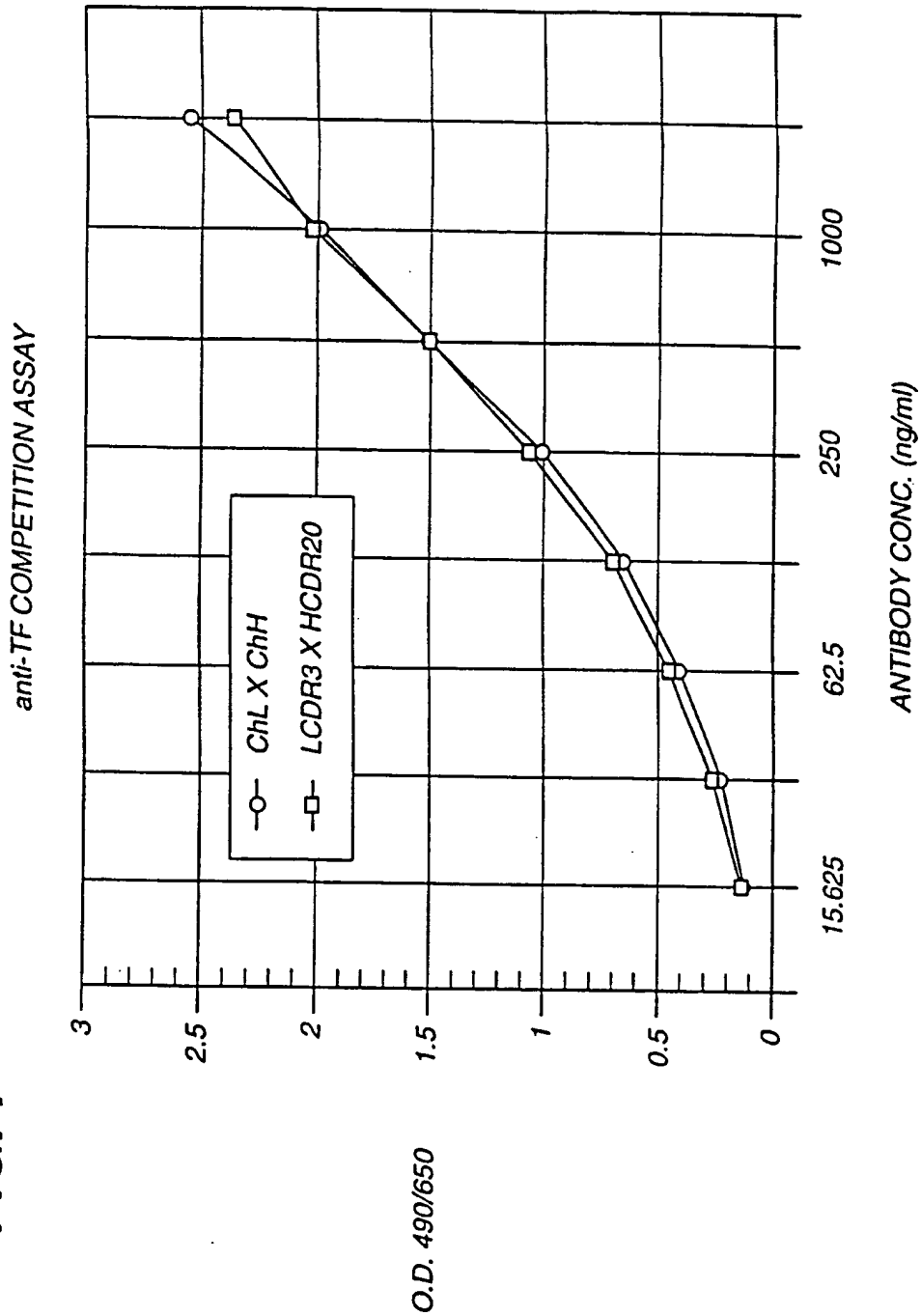
**FIG. 5 O**

7830														
	CGA	TCG	ACT	CTA	GAG	GAT	CGA	TCC	CCG	GGC	GAG	CTC	G	
	GCT	AGC	TGA	GAT	CTC	CTA	GCT	AGG	GGC	CCG	CTC	GAG	C	



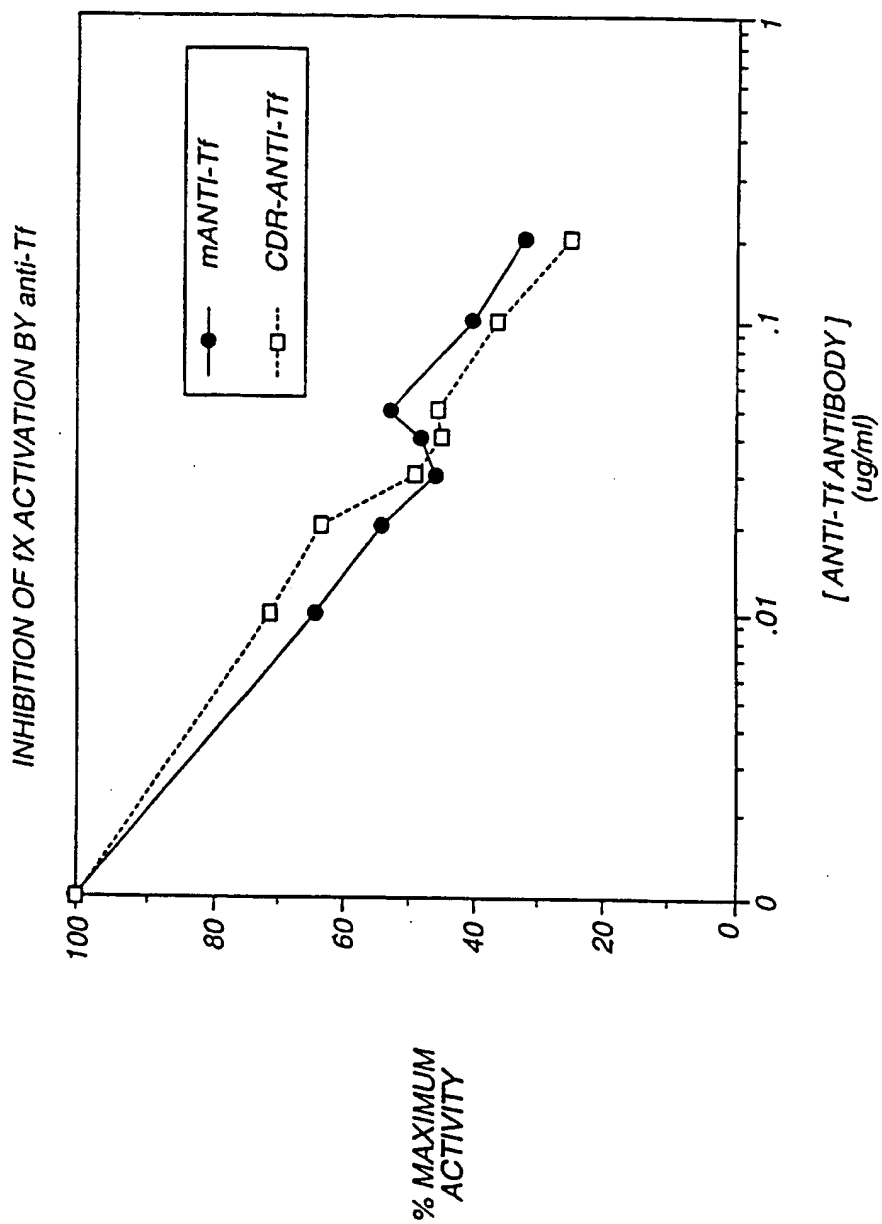
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**FIG. 7**



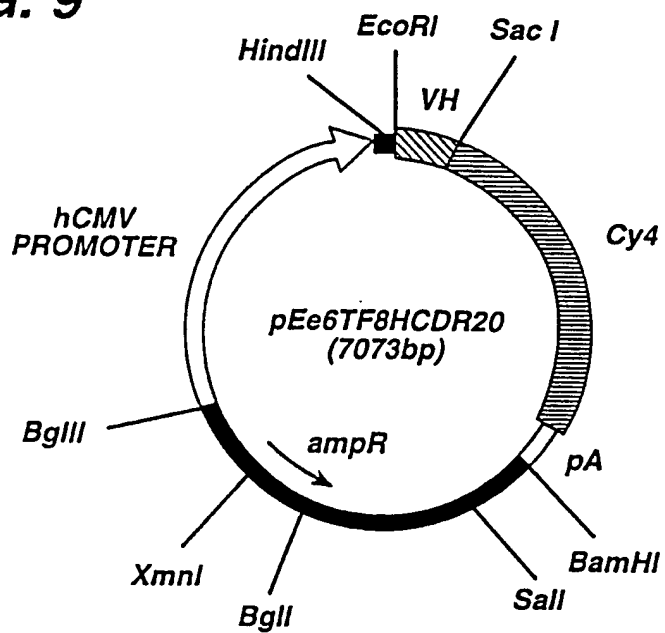
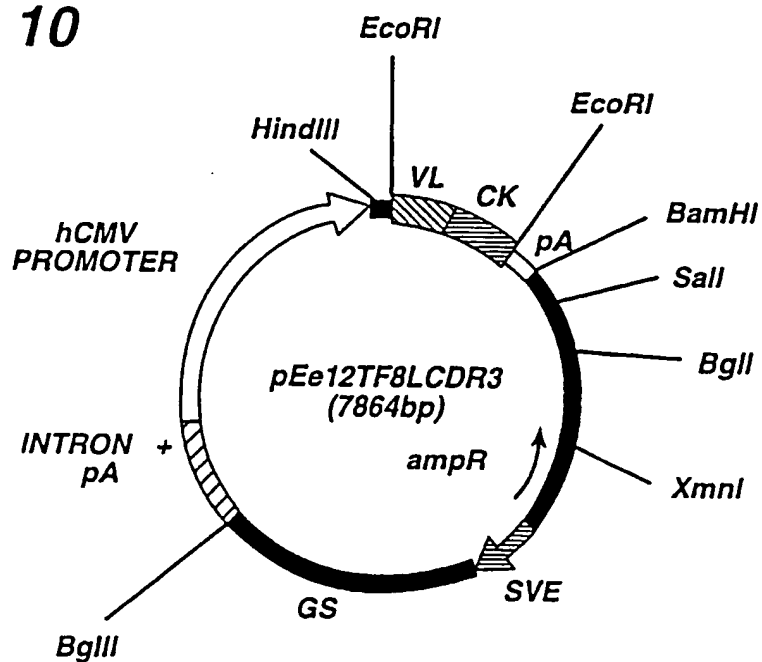
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**FIG. 8**





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**FIG. 9****FIG. 10**

PCT/US 96/09287

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 6 C12N15/13 C07K16/36 C07K16/46 A61K39/395 //C12N5/10,  
C12N15/85

### B. FIELDS SEARCHED

IPC 6 C12N C07K A61K

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
------------	--	-----------------------

Y	WO 91 09968 A (CELLTECH LIMITED) 11 July 1991 see examples see claims	1-37
Y	WO 88 07543 A (SCRIPPS CLINIC AND RESEARCH FOUNDATION) 6 October 1988 see claims	1-37
A	WO 94 11029 A (THE SCRIPPS RESEARCH INSTITUTE ET AL.) 26 May 1994 see claims	1-37
A	WO 94 05328 A (THE SCRIPPS RESEARCH INSTITUTE) 17 March 1994 see examples see claims	1-37

-/-

☒ Patent family members are listed in annex.

- \* "A" document defining the general state of the art which is not considered to be of particular relevance
- \* "E" earlier document but published on or after the international filing date
- \* "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \* "O" document referring to an oral disclosure, use, exhibition or other means
- \* "P" document published prior to the international filing date but later than the priority date claimed

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*& document member of the same patent family

15 October 1996

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Fax: (+31-70) 340-3016

Nooij, F

# INTERNATIONAL SEARCH REPORT

International Application No

PC1/US 96/09287

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>JOURNAL OF CRYSTAL GROWTH, vol. 122, no. 1-4, August 1992, AMSTERDAM, NL, pages 253-264, XP002015918 W. RUF ET AL.: "Purification, sequence and crystallization of an anti-tissue factor Fab and its use for the crystallization of tissue factor." see abstract see table 1</p> <p style="text-align: center;">-----</p>	1-37

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 96/09287

**Box I** Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 31-35  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claims 31-35 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II** Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PC1/US 96/09287

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Information on patent family members

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PC1/US 96/09287

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